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(54) **BRASSICA PLANTS YIELDING OILS WITH A LOW ALPHA LINOLENIC ACID CONTENT**

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(51) **Int. Cl.**

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**C12N 9/00** (2006.01)  
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(52) **U.S. Cl.**

CPC ..... **A01H 5/10** (2013.01); **C12N 9/0083** (2013.01); **C12N 15/8247** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

*Brassica* plants producing oils with a low alpha-linolenic acid content and methods for producing such plants are described.

**6 Claims, 20 Drawing Sheets**

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86a3x338												
		30	20	30	40	30	30	30	30	30	30	30
1,994,86a3x338~2, seqq												
1,994,86a3x338~3, seqq												
86a3x338, seqq												
86a3x349												
		80	100	110	120	130	140	150	160			
1,994,86a3x349~2, seqq												
1,994,86a3x349~3, seqq												
86a3x349, seqq												
86a3x359												
		170	180	190	200	210	220	230	240			
1,994,86a3x359~2, seqq												
1,994,86a3x359~3, seqq												
86a3x359, seqq												
86a3x419												
		250	260	270	280	290	300	310	320			
1,994,86a3x419~2, seqq												
1,994,86a3x419~3, seqq												
86a3x419, seqq												
86a3x429												
		310	340	350	360	370	380	390	400			
1,994,86a3x429~2, seqq												
1,994,86a3x429~3, seqq												
86a3x429, seqq												

FIGURE 1

1804 Bone 33~2, 889	GTCGGCTGCGCTGTTGATGCCAGACGTTTGCAACGGCGGTG	307
1804 Bone 33~2, 889	GGTGCCTGGCTCCATTCGGGCGGCAGAACTTCTCCTGCAC	387
1804 Bone 33~2, 889	GTCGCCTGGCTCCATTCGGGCGGCAGAACTTCTCCTGCAC	609
Majority	TCCTGGCTGGCATGGTGGATGTTGCTGGGGGCTGTCCTG	
	430 420 430 440 430 440 473 430	430
1804 Bone 33~2, 889	TCTTCAATTTCACCTGGTGAATATTTGAAATGCGCTGTC	287
1804 Bone 33~2, 889	TCTTCAATTTCACCTGGTGAATATTTGAAATGCGCTGTC	387
1804 Bone 33~2, 889	TCTTCAATTTCACCTGGTGAATATTTGAAATGCGCTGTC	680
Majority	TTGAGTCATGTTGGGTTGACGGATTTCTTCAGTTTC	
	430 309 510 369 530 368 530 369	369
1804 Bone 33~2, 889	TTGAGTCATGTTGGGTTGACGGATTTCTTCAGTTTC	467
1804 Bone 33~2, 889	TTGAGTCATGTTGGGTTGACGGATTTCTTCAGTTTC	687
1804 Bone 33~2, 889	TTGAGTCATGTTGGGTTGACGGATTTCTTCAGTTTC	909
Majority	TGGGGTGGACCCCGGGGAGGGGGGGGGGGGGGGGGG	
	916 910 930 930 916 916 916 916	916
1804 Bone 33~2, 889	TGGGGTGGACCCCGGGGAGGGGGGGGGGGGGGGGGG	347
1804 Bone 33~2, 889	TGGGGTGGACCCCGGGGAGGGGGGGGGGGGGGGGGG	649
Majority	TTGGAAACAGAACAGAACAGAACAGAACAGAACAG	
	630 630 630 630 630 630 713 720	630
1804 Bone 33~2, 889	TTGGAAACAGAACAGAACAGAACAGAACAGAACAG	627
1804 Bone 33~2, 889	TTGGAAACAGAACAGAACAGAACAGAACAGAACAG	720
Majority	TGGGGTGGACCCCGGGGAGGGGGGGGGGGGGGGGGG	
	736 740 750 740 730 740 740 730	730
1804 Bone 33~2, 889	TGGGGTGGACCCCGGGGAGGGGGGGGGGGGGGGGGG	797
1804 Bone 33~2, 889	TGGGGTGGACCCCGGGGAGGGGGGGGGGGGGGGGGG	797

FIGURE 1 (CONT.)

848 4438, seqq.	Y3C877CC08A2C78336A08V7TAAAGAATCCTAACCTTCATGTTTAAAGGTGTAAACGGTAAAGTC	869
888 3263, seqq	TAAATAAAATGCGGATCAAGTAAATGATATAATGAAATATGGAAATATAATTAATTATAATGAAATATAATGA	886
190 4 Befp338~2, seqq	ATG 810 820 830 840 850 860 870 880	887
346 201 Nov 4338~2, seqq	Y3C877CC08A2C78336A08V7TAAAGAATCCTAACCTTCATGTTTAAAGGTGTAAACGGTAAAGTC	887
818 4438, seqq	TAAATAAAATGCGGATCAAGTAAATGATATAATGAAATATGGAAATATAATTAATTATAATGAAATATAATGA	888
888 3264 19	Y3C877CC08A2C78336A08V7TAAAGAATCCTAACCTTCATGTTTAAAGGTGTAAACGGTAAAGTC	889
	889 889 890 891 892 893 894 895	890
190 4 Befp338~2, seqq	AAGGGAATGCTATGAACTTCATGATATGATATGGATGATGATATGGATGATGATATGGATGATGATATGGAT	891
346 201 Nov 4338~2, seqq	AAGGGAATGCTATGAACTTCATGATATGATATGGATGATGATATGGATGATGATATGGATGATGATATGGAT	892
818 4438, seqq	AAGGGAATGCTATGAACTTCATGATATGATATGGATGATGATATGGATGATGATATGGATGATGATATGGAT	893
888 3264 19	Y3C877CC08A2C78336A08V7TAAAGAATCCTAACCTTCATGTTTAAAGGTGTAAACGGTAAAGTC	894
	894 895 896 897 898 899 899 899	895
190 4 Befp338~2, seqq	GATCTGGTGAACTGGCTTGTCTTCTTCTTCCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT	896
346 201 Nov 4338~2, seqq	GATCTGGTGAACTGGCTTGTCTTCTTCTTCCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT	897
818 4438, seqq	GATCTGGTGAACTGGCTTGTCTTCTTCTTCCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT	898
888 3264 19	Y3C877CC08A2C78336A08V7TAAAGAATCCTAACCTTCATGTTTAAAGGTGTAAACGGTAAAGTC	899
	899 900 900 900 900 900 900 900	900
190 4 Befp338~2, seqq	GGTTTTCT	901
346 201 Nov 4338~2, seqq	GGTTTTCT	902
818 4438, seqq	GGTTTTCT	903
888 3264 19	Y3C877CC08A2C78336A08V7TAAAGAATCCTAACCTTCATGTTTAAAGGTGTAAACGGTAAAGTC	904
	904 905 906 907 907 908 908 908	905
190 4 Befp338~2, seqq	CCCT	906
346 201 Nov 4338~2, seqq	CCCT	907
818 4438, seqq	CCCT	908
888 3264 19	Y3C877CC08A2C78336A08V7TAAAGAATCCTAACCTTCATGTTTAAAGGTGTAAACGGTAAAGTC	909
	909 910 911 911 912 912 912 912	910

00030618					
1310	1220	1230	1240	1250	1260
1304 808-38-2, seqq	00030618-2, seqq	00030618-38-2, seqq	00030618-38-3, seqq	00030618-38-4, seqq	00030618-38-5, seqq
1305 1 Buff-38-2, seqq	1306 1 Buff-38-3, seqq	1307 1 Buff-38-4, seqq	1308 1 Buff-38-5, seqq	1309 1 Buff-38-6, seqq	1310 1 Buff-38-7, seqq
<i>Majority</i>					
1310	1320	1330	1340	1350	1360
1304 808-38-2, seqq	00030618-2, seqq	00030618-38-2, seqq	00030618-38-3, seqq	00030618-38-4, seqq	00030618-38-5, seqq
1305 1 Buff-38-2, seqq	1306 1 Buff-38-3, seqq	1307 1 Buff-38-4, seqq	1308 1 Buff-38-5, seqq	1309 1 Buff-38-6, seqq	1310 1 Buff-38-7, seqq
<i>Minority</i>					
1310	1320	1330	1340	1350	1360
1304 808-38-2, seqq	00030618-2, seqq	00030618-38-2, seqq	00030618-38-3, seqq	00030618-38-4, seqq	00030618-38-5, seqq
1305 1 Buff-38-2, seqq	1306 1 Buff-38-3, seqq	1307 1 Buff-38-4, seqq	1308 1 Buff-38-5, seqq	1309 1 Buff-38-6, seqq	1310 1 Buff-38-7, seqq
<i>Majority</i>					
1310	1320	1330	1340	1350	1360
1304 808-38-2, seqq	00030618-2, seqq	00030618-38-2, seqq	00030618-38-3, seqq	00030618-38-4, seqq	00030618-38-5, seqq
1305 1 Buff-38-2, seqq	1306 1 Buff-38-3, seqq	1307 1 Buff-38-4, seqq	1308 1 Buff-38-5, seqq	1309 1 Buff-38-6, seqq	1310 1 Buff-38-7, seqq
<i>Minority</i>					
1310	1320	1330	1340	1350	1360
1304 808-38-2, seqq	00030618-2, seqq	00030618-38-2, seqq	00030618-38-3, seqq	00030618-38-4, seqq	00030618-38-5, seqq
1305 1 Buff-38-2, seqq	1306 1 Buff-38-3, seqq	1307 1 Buff-38-4, seqq	1308 1 Buff-38-5, seqq	1309 1 Buff-38-6, seqq	1310 1 Buff-38-7, seqq
<i>Majority</i>					
1310	1320	1330	1340	1350	1360
1304 808-38-2, seqq	00030618-2, seqq	00030618-38-2, seqq	00030618-38-3, seqq	00030618-38-4, seqq	00030618-38-5, seqq
1305 1 Buff-38-2, seqq	1306 1 Buff-38-3, seqq	1307 1 Buff-38-4, seqq	1308 1 Buff-38-5, seqq	1309 1 Buff-38-6, seqq	1310 1 Buff-38-7, seqq

FIGURE 3 (CONT.)

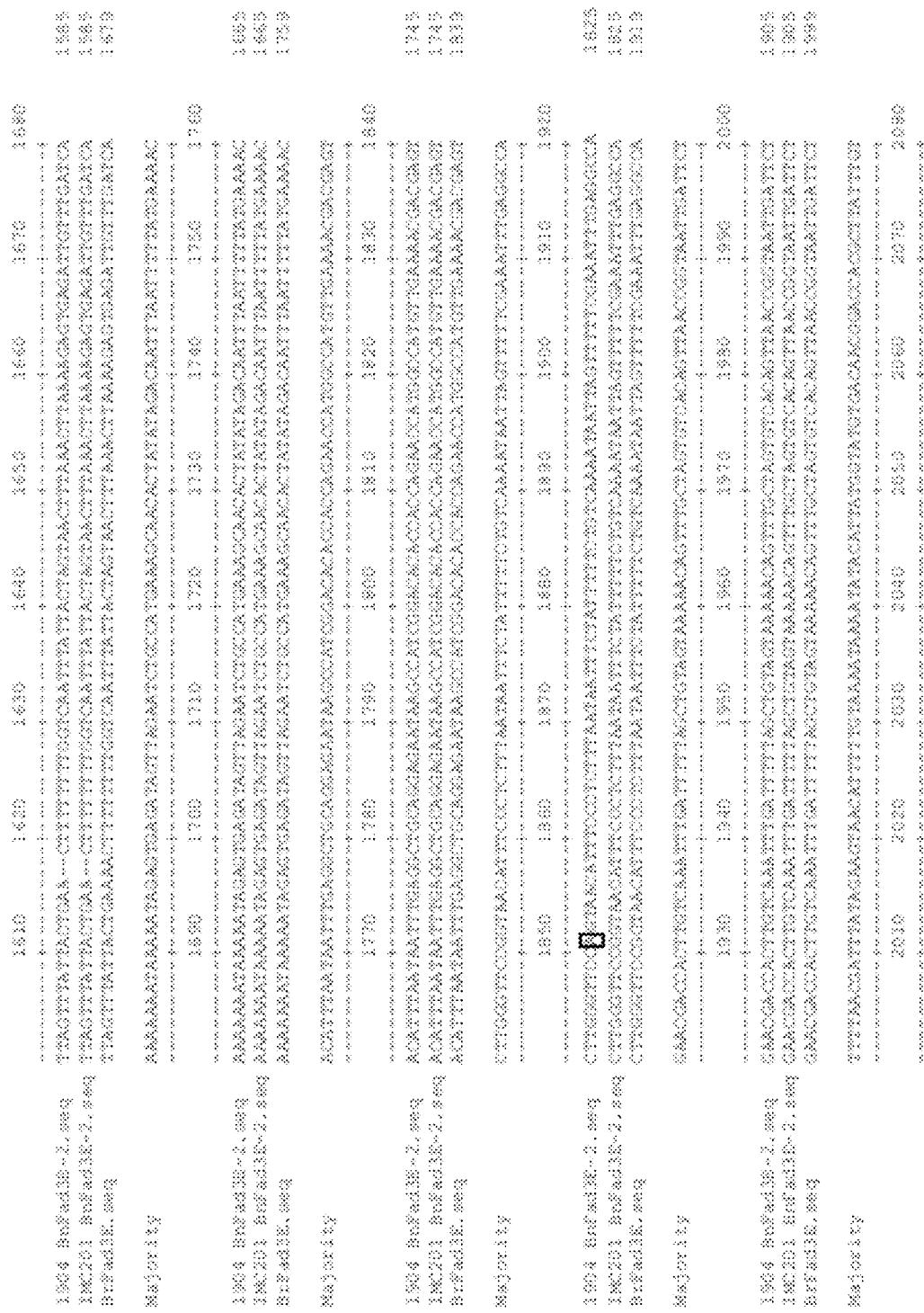


FIGURE 1 (CONT.)

1304 Brefad38-2, seqq 38C01 Brefad38-2, seqq 837438, seqq	TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG	1303 1303 2079	2130 2130 2130	2140 2140 2140	2150 2150 2150	2160 2160 2160
Molarity	TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG	2179	2180	2180	2210	2220
1304 Brefad38-2, seqq 38C01 Brefad38-2, seqq 837438, seqq	TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG	2143 2143 2143	2143 2143 2143	2143 2143 2143	2143 2143 2143	2143 2143 2143
Molarity	TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG	2230	2235	2235	2235	2235
1304 Brefad38-2, seqq 38C01 Brefad38-2, seqq 837438, seqq	TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG	2223 2223 2223	2223 2223 2223	2223 2223 2223	2223 2223 2223	2223 2223 2223
Molarity	TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG	2330	2340	2340	2340	2340
1304 Brefad38-2, seqq 38C01 Brefad38-2, seqq 837438, seqq	TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG	2323 2323 2323	2323 2323 2323	2323 2323 2323	2323 2323 2323	2323 2323 2323
Molarity	TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG	2410	2420	2420	2440	2450
1304 Brefad38-2, seqq 38C01 Brefad38-2, seqq 837438, seqq	TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG	2480 2480 2480	2490 2490 2490	2490 2490 2490	2490 2490 2490	2490 2490 2490
1304 Brefad38-2, seqq 38C01 Brefad38-2, seqq 837438, seqq	TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG TTTAACTTTAGAATTCACATTGTTTCTTAAATAAACTTAACCAATTGTTTACACCGGGCTACGTTTGTGTTG	2585 2585 2585	2585 2585 2585	2585 2585 2585	2585 2585 2585	2585 2585 2585

FIGURE 1 (CONT.)



Majority			
TACAGCTTCAATTTGCTTCATACTTTGGGTAACTTCCATTCACTTGCCTT	2830	2986	2916
CTTAACATTTCAATTTGCTTCATACTTTGGGTAACTTCCATTCACTTGCCTT	2986	2916	2830
TACAGCTTCAATTTGCTTCATACTTTGGGTAACTTCCATTCACTTGCCTT	3004	3048~2, seqq	3020~1 Before d32~2, seqq
TTACATTTCAATTTGCTTCATACTTTGGGTAACTTCCATTCACTTGCCTT	3020~1 Before d32~2, seqq	3020~1 Before d32~2, seqq	3020~1 Before d32~2, seqq
Minority			
AAAGAAAATCCTTCACTTTCTTCACTTCCTTCACTTCCTTCACTTCCTT	2919	2980	2950
AAAGAAAATCCTTCACTTTCTTCACTTCCTTCACTTCCTTCACTTCCTT	3004	3048~2, seqq	3020~1 Before d32~2, seqq
AAAGAAAATCCTTCACTTTCTTCACTTCCTTCACTTCCTTCACTTCCTT	3020~1 Before d32~2, seqq	3020~1 Before d32~2, seqq	3020~1 Before d32~2, seqq
Majority			
CTTACATTTCAATTTGCTTCATACTTTGGGTAACTTCCATTCACTTGCCTT	3030	3089	3078
CTTACATTTCAATTTGCTTCATACTTTGGGTAACTTCCATTCACTTGCCTT	3048	3109	3098
CTTACATTTCAATTTGCTTCATACTTTGGGTAACTTCCATTCACTTGCCTT	3066	3140	3130
CTTACATTTCAATTTGCTTCATACTTTGGGTAACTTCCATTCACTTGCCTT	3084	3164~2, seqq	3100~1 Before d32~2, seqq
CTTACATTTCAATTTGCTTCATACTTTGGGTAACTTCCATTCACTTGCCTT	3104	3184~2, seqq	3120~1 Before d32~2, seqq
Minority			
CATACTTTGATTTGGGTAACTTCCATTCACTTGCCTT	3130	3186	3166
CATACTTTGATTTGGGTAACTTCCATTCACTTGCCTT	3148	3206	3186
CATACTTTGATTTGGGTAACTTCCATTCACTTGCCTT	3166	3224	3186
CATACTTTGATTTGGGTAACTTCCATTCACTTGCCTT	3184	3243	3186
CATACTTTGATTTGGGTAACTTCCATTCACTTGCCTT	3203	3261	3186
CATACTTTGATTTGGGTAACTTCCATTCACTTGCCTT	3221	3279	3186
CATACTTTGATTTGGGTAACTTCCATTCACTTGCCTT	3240	3298	3186
CATACTTTGATTTGGGTAACTTCCATTCACTTGCCTT	3258	3316	3186
CATACTTTGATTTGGGTAACTTCCATTCACTTGCCTT	3276	3334	3186
CATACTTTGATTTGGGTAACTTCCATTCACTTGCCTT	3294	3352	3186
CATACTTTGATTTGGGTAACTTCCATTCACTTGCCTT	3312	3370	3186
CATACTTTGATTTGGGTAACTTCCATTCACTTGCCTT	3330	3388	3186

FIGURE 1 (CONT.)



1304 8seqd30>2, seq-q ACCCTGAAAGCAGAATCGTGGTCAACTGGTGGGCGAGGTTTGACGAGCTTAAGGAAAGCTTACGCTGGA  
1380 301 8seqd33>2, seq-q AGCTGAAAGCAGAATCGTGGTCAACTGGTGGGCGAGGTTTGACGAGCTTAAGGAAAGCTTACGCTGGA  
8.6seqd30, seq-q AGCTGAAAGCAGAATCGTGGTCAACTGGTGGGCGAGGTTTGACGAGCTTAAGGAAAGCTTACGCTGGA  
8.6seqd33, seq-q AGCTGAAAGCAGAATCGTGGTCAACTGGTGGGCGAGGTTTGACGAGCTTAAGGAAAGCTTACGCTGGA  
  
8830x138  
TGACCTTGCCTGGTCAACTGGTGGGCGAGGTTTGACGAGCTTAAGGAAAGCTTACGCTGGA  
3776 3780 3784 3806 3810 3814 3828 3830  
3602  
1304 8seqd30>2, seq-q TGACCTTGCCTGGTCAACTGGTGGGCGAGGTTTGACGAGCTTAAGGAAAGCTTACGCTGGA  
138201 8seqd33>2, seq-q TGACCTTGCCTGGTCAACTGGTGGGCGAGGTTTGACGAGCTTAAGGAAAGCTTACGCTGGA  
8.6seqd30, seq-q TGACCTTGCCTGGTCAACTGGTGGGCGAGGTTTGACGAGCTTAAGGAAAGCTTACGCTGGA  
8.6seqd33, seq-q TGACCTTGCCTGGTCAACTGGTGGGCGAGGTTTGACGAGCTTAAGGAAAGCTTACGCTGGA

FIGURE 1 (CONT.)

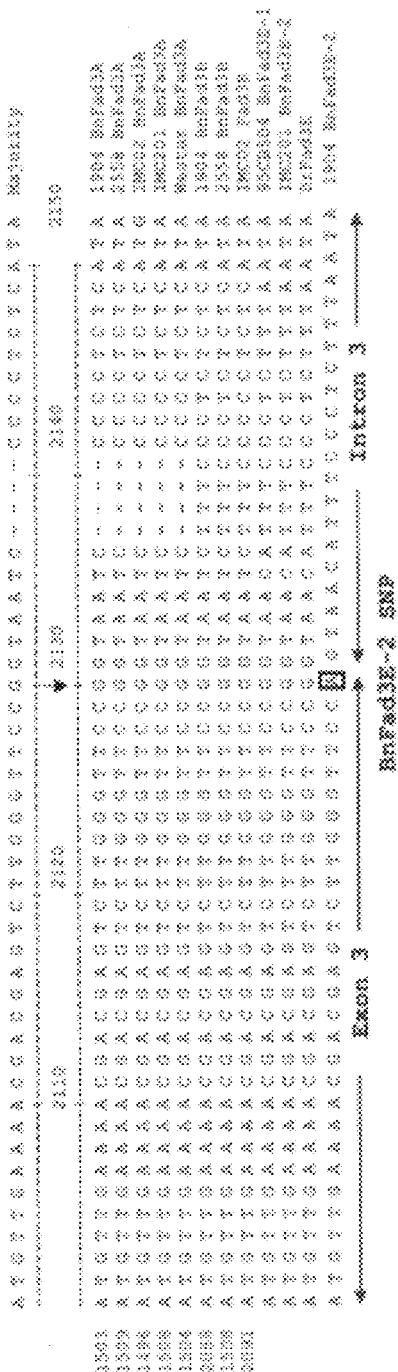


FIGURE 2

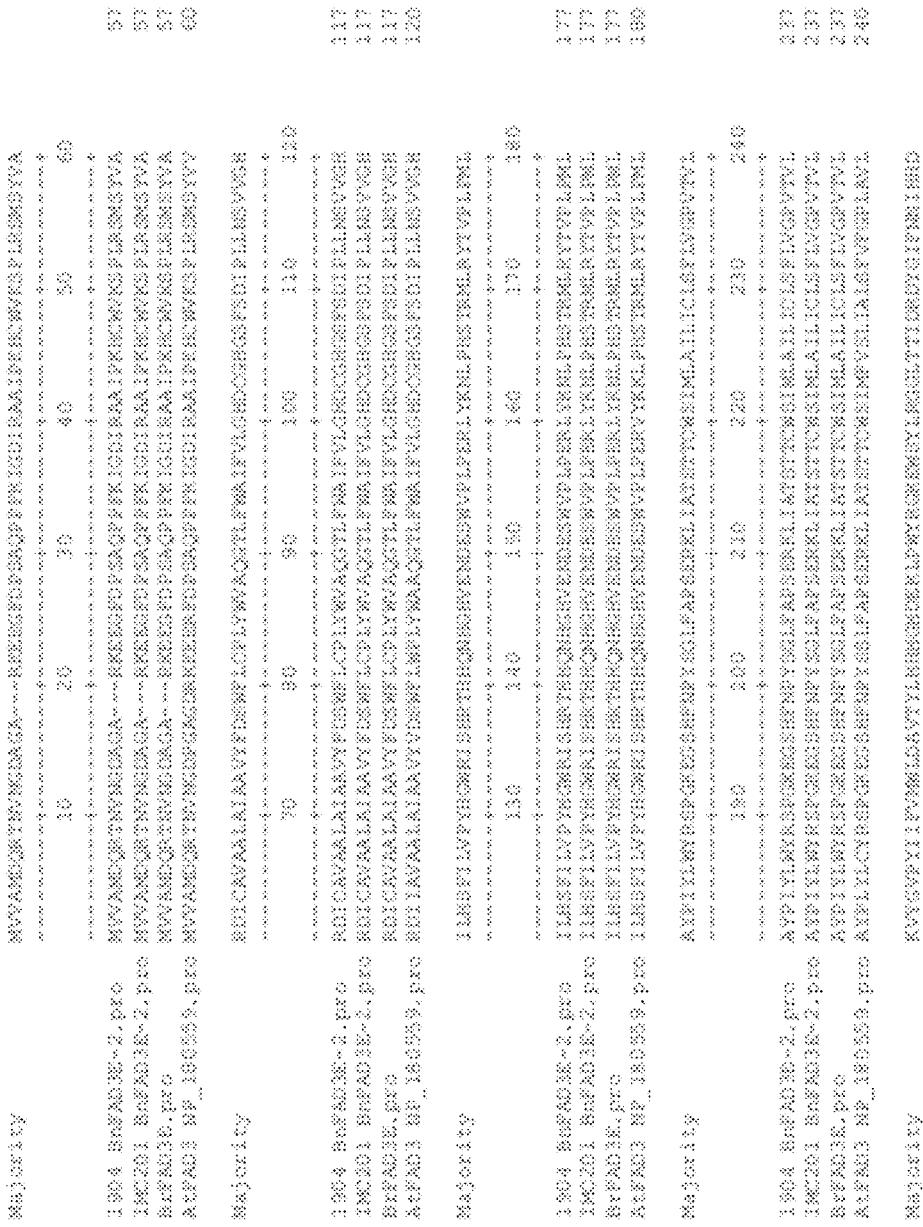


FIGURE 3



seqid 1  
 AACGCT AACCGATTTTACGGCCACAGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 10 20 30 40 50 60 70 80  
 AACGCT TAAACAAATTTCCTGCGCTACGTTTGTCAACATGTTTACGGCCACAGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 AACGCT AACGAATTTCATACGGCCACAGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 seqid 2  
 GATTTTTT TTTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 80 100 120 140 160 180 200  
 GATTTTTT TTTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 GATTTTTT TTTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 seqid 3  
 ATACACGTTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 100 120 140 160 180 200 220 240  
 ATACACGTTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 ATACACGTTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 ATACACGTTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 seqid 4  
 TTTTGTATTTTAAATTTTTTGTGGCACTTATGCGCTACGGCCACAGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 80 100 120 140 160 180 200 220 240 260  
 TTTTGTATTTTAAATTTTTTGTGGCACTTATGCGCTACGGCCACAGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 TTTTGTATTTTAAATTTTTTGTGGCACTTATGCGCTACGGCCACAGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 TTTTGTATTTTAAATTTTTTGTGGCACTTATGCGCTACGGCCACAGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 seqid 5  
 TCTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 80 100 120 140 160 180 200 220 240  
 TCTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 TCTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 TCTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 seqid 6  
 TCTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 100 120 140 160 180 200 220 240 260  
 TCTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 TCTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 TCTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 seqid 7  
 ATTCGCGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 80 100 120 140 160 180 200 220 240  
 ATTCGCGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 ATTCGCGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 ATTCGCGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 seqid 8  
 CCTTAACGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 80 100 120 140 160 180 200 220 240  
 CCTTAACGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 CCTTAACGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 CCTTAACGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 seqid 9  
 GATGGGCGGCGGCTTGTTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 80 100 120 140 160 180 200 220 240  
 GATGGGCGGCGGCTTGTTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 GATGGGCGGCGGCTTGTTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 seqid 10  
 TCTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 80 100 120 140 160 180 200 220 240  
 TCTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 TCTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 TCTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 seqid 11  
 ATTCGCGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 80 100 120 140 160 180 200 220 240  
 ATTCGCGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 ATTCGCGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 ATTCGCGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 seqid 12  
 GATGGGCGGCGGCTTGTTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 80 100 120 140 160 180 200 220 240  
 GATGGGCGGCGGCTTGTTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 GATGGGCGGCGGCTTGTTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG  
 GATGGGCGGCGGCTTGTTTACGGCTTATGTTTGCACAAATTGTTAATGCGCAGAACATGGCAAAATGTTGTTTGAG

Repeat 1: AATTAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG  
 890 891 892 893 894 895 896 897 898

899GCTTGATTTTAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 899  
 1900 GCTTGATTTTAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 899  
 1901 GCTTGATTTTAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 899

Repeat 2: TAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG  
 902 903 904 905 906 907 908 909 910

911TAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 911  
 1912TAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 912  
 1913TAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 913

Repeat 3: GCTTGATTTTAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG  
 1914 1915 1916 1917 1918 1919 1920 1921 1922

1923GCTTGATTTTAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1923  
 1924 GCTTGATTTTAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1924  
 1925 GCTTGATTTTAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1925

Repeat 4: TAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG  
 1926 1927 1928 1929 1930 1931 1932 1933 1934

1935TAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1935  
 1936 TAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1936  
 1937 TAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1937

Repeat 5: AAATGCTTAAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG  
 1938 1939 1940 1941 1942 1943 1944 1945 1946

1947AAATGCTTAAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1947  
 1948 AAATGCTTAAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1948  
 1949 AAATGCTTAAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1949

Repeat 6: CACATGAACTCTAATATGAACTCTGGCTATCGAAGGAACTCTGTTGGGCTATGG  
 1950 1951 1952 1953 1954 1955 1956 1957 1958

1959CACATGAACTCTAATATGAACTCTGGCTATCGAAGGAACTCTGTTGGGCTATGG 1959  
 1960 CACATGAACTCTAATATGAACTCTGGCTATCGAAGGAACTCTGTTGGGCTATGG 1960  
 1961 CACATGAACTCTAATATGAACTCTGGCTATCGAAGGAACTCTGTTGGGCTATGG 1961

Repeat 7: GCTTGATTTTAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG  
 1962 1963 1964 1965 1966 1967 1968 1969 1970

1971GCTTGATTTTAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1971  
 1972 GCTTGATTTTAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1972  
 1973 GCTTGATTTTAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1973

Repeat 8: TAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG  
 1974 1975 1976 1977 1978 1979 1980 1981 1982

1983TAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1983  
 1984 TAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1984  
 1985 TAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1985

Repeat 9: AAATGCTTAAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG  
 1986 1987 1988 1989 1990 1991 1992 1993 1994

1995AAATGCTTAAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1995  
 1996 AAATGCTTAAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1996  
 1997 AAATGCTTAAACTATGAAATCTAAGATCTACATCATCAGTCACGTCAACCTGAACTG 1997

Repeat 10: CACATGAACTCTAATATGAACTCTGGCTATCGAAGGAACTCTGTTGGGCTATGG  
 1998 1999 2000 2001 2002 2003 2004 2005 2006

2007CACATGAACTCTAATATGAACTCTGGCTATCGAAGGAACTCTGTTGGGCTATGG 2007  
 2008 CACATGAACTCTAATATGAACTCTGGCTATCGAAGGAACTCTGTTGGGCTATGG 2008  
 2009 CACATGAACTCTAATATGAACTCTGGCTATCGAAGGAACTCTGTTGGGCTATGG 2009

FIGURE 4 (CONT.)

**Species:** *S. lutea* L. *S. longiloba* L.

	1200	1300	1400	1500	1600	1700	1800	1900
<i>S. lutea</i> L. (spec. g. 2000)	TTT	CCCT	GGG	TG	AC	AT	CG	TG
<i>S. longiloba</i> L. (spec. g. 2000)	TC	AT	TT	TC	GT	CT	AG	TT
<i>S. longiloba</i> L. (spec. g. 2000)	TTT	TC	AT	TT	TC	GT	CT	AG
<b>Species:</b> <i>S. lutea</i> L. <i>S. longiloba</i> L.	GGG	TC	AT	TT	TC	GT	CT	AG
<i>S. lutea</i> L. (spec. g. 2000)	TTT	CCCT	GGG	TG	AC	AT	CG	TG
<i>S. longiloba</i> L. (spec. g. 2000)	TC	AT	TT	TC	GT	CT	AG	TT
<i>S. longiloba</i> L. (spec. g. 2000)	TTT	TC	AT	TT	TC	GT	CT	AG
<b>Species:</b> <i>S. lutea</i> L. <i>S. longiloba</i> L.	GGG	TC	AT	TT	TC	GT	CT	AG
<i>S. lutea</i> L. (spec. g. 2000)	TTT	CCCT	GGG	TG	AC	AT	CG	TG
<i>S. longiloba</i> L. (spec. g. 2000)	TC	AT	TT	TC	GT	CT	AG	TT
<i>S. longiloba</i> L. (spec. g. 2000)	TTT	TC	AT	TT	TC	GT	CT	AG
<b>Species:</b> <i>S. lutea</i> L. <i>S. longiloba</i> L.	GGG	TC	AT	TT	TC	GT	CT	AG
<i>S. lutea</i> L. (spec. g. 2000)	TTT	CCCT	GGG	TG	AC	AT	CG	TG
<i>S. longiloba</i> L. (spec. g. 2000)	TC	AT	TT	TC	GT	CT	AG	TT
<i>S. longiloba</i> L. (spec. g. 2000)	TTT	TC	AT	TT	TC	GT	CT	AG
<b>Species:</b> <i>S. lutea</i> L. <i>S. longiloba</i> L.	GGG	TC	AT	TT	TC	GT	CT	AG
<i>S. lutea</i> L. (spec. g. 2000)	TTT	CCCT	GGG	TG	AC	AT	CG	TG
<i>S. longiloba</i> L. (spec. g. 2000)	TC	AT	TT	TC	GT	CT	AG	TT
<i>S. longiloba</i> L. (spec. g. 2000)	TTT	TC	AT	TT	TC	GT	CT	AG
<b>Species:</b> <i>S. lutea</i> L. <i>S. longiloba</i> L.	GGG	TC	AT	TT	TC	GT	CT	AG
<i>S. lutea</i> L. (spec. g. 2000)	TTT	CCCT	GGG	TG	AC	AT	CG	TG
<i>S. longiloba</i> L. (spec. g. 2000)	TC	AT	TT	TC	GT	CT	AG	TT
<i>S. longiloba</i> L. (spec. g. 2000)	TTT	TC	AT	TT	TC	GT	CT	AG
<b>Species:</b> <i>S. lutea</i> L. <i>S. longiloba</i> L.	GGG	TC	AT	TT	TC	GT	CT	AG
<i>S. lutea</i> L. (spec. g. 2000)	TTT	CCCT	GGG	TG	AC	AT	CG	TG
<i>S. longiloba</i> L. (spec. g. 2000)	TC	AT	TT	TC	GT	CT	AG	TT
<i>S. longiloba</i> L. (spec. g. 2000)	TTT	TC	AT	TT	TC	GT	CT	AG
<b>Species:</b> <i>S. lutea</i> L. <i>S. longiloba</i> L.	GGG	TC	AT	TT	TC	GT	CT	AG
<i>S. lutea</i> L. (spec. g. 2000)	TTT	CCCT	GGG	TG	AC	AT	CG	TG
<i>S. longiloba</i> L. (spec. g. 2000)	TC	AT	TT	TC	GT	CT	AG	TT
<i>S. longiloba</i> L. (spec. g. 2000)	TTT	TC	AT	TT	TC	GT	CT	AG
<b>Species:</b> <i>S. lutea</i> L. <i>S. longiloba</i> L.	GGG	TC	AT	TT	TC	GT	CT	AG
<i>S. lutea</i> L. (spec. g. 2000)	TTT	CCCT	GGG	TG	AC	AT	CG	TG
<i>S. longiloba</i> L. (spec. g. 2000)	TC	AT	TT	TC	GT	CT	AG	TT
<i>S. longiloba</i> L. (spec. g. 2000)	TTT	TC	AT	TT	TC	GT	CT	AG
<b>Species:</b> <i>S. lutea</i> L. <i>S. longiloba</i> L.	GGG	TC	AT	TT	TC	GT	CT	AG
<i>S. lutea</i> L. (spec. g. 2000)	TTT	CCCT	GGG	TG	AC	AT	CG	TG
<i>S. longiloba</i> L. (spec. g. 2000)	TC	AT	TT	TC	GT	CT	AG	TT
<i>S. longiloba</i> L. (spec. g. 2000)	TTT	TC	AT	TT	TC	GT	CT	AG

FIGURE 4 (CONT.)

FIGURE 4 (CONT.)

**Sequence 1**

ATTTCTCTGAAACCGTTTATTTAAGAAACACCAAGGGTCAGAGTCAGCTTGGACATT  
3' 5' 3' 5' 3' 5' 3' 5' 3' 5' 3' 5' 3' 5' 3' 5' 3' 5'  
AGCTCCTTAAACGTTTATTTAAGAAACACCAAGGGTCAGAGTCAGCTTGGACATT  
ATTTCTCTGAAACCGTTTATTTAAGAAACACCAAGGGTCAGAGTCAGCTTGGACATT  
**Sequence 2**

TCACAGGAAAATCTCTTCCCTGAAAAGGCGGCTTCAGCTCTCAGAGTCAGCTTGGACATT  
3' 5' 3' 5' 3' 5' 3' 5' 3' 5' 3' 5' 3' 5' 3' 5'  
TCACAGGAAAATCTCTTCCCTGAAAAGGCGGCTTCAGCTCTCAGAGTCAGCTTGGACATT  
TCACAGGAAAATCTCTTCCCTGAAAAGGCGGCTTCAGCTCTCAGAGTCAGCTTGGACATT  
**Sequence 3**

TTTGTCTTCAATCTTAAACGTTTATTTAAGAAACACCAAGGGTCAGAGTCAGCTTGGACATT  
3' 5' 3' 5' 3' 5' 3' 5' 3' 5' 3' 5' 3' 5' 3' 5'  
TTTGTCTTCAATCTTAAACGTTTATTTAAGAAACACCAAGGGTCAGAGTCAGCTTGGACATT  
TTTGTCTTCAATCTTAAACGTTTATTTAAGAAACACCAAGGGTCAGAGTCAGCTTGGACATT  
TTTGTCTTCAATCTTAAACGTTTATTTAAGAAACACCAAGGGTCAGAGTCAGCTTGGACATT  
**Sequence 4**

CAGAACGAAACTTTTCAATGAACTTTCAGACGTTCTTCACAGTTGGACATT  
3' 5' 3' 5' 3' 5' 3' 5' 3' 5' 3' 5'  
CAGAACGAAACTTTTCAATGAACTTTCAGACGTTCTTCACAGTTGGACATT  
CAGAACGAAACTTTTCAATGAACTTTCAGACGTTCTTCACAGTTGGACATT  
CAGAACGAAACTTTTCAATGAACTTTCAGACGTTCTTCACAGTTGGACATT  
**Sequence 5**

TTTTTCTCTCATGAAACAACTTACATGAACTTATTTCTCTTCTTCACAGTTGGACATT  
3' 5' 3' 5' 3' 5' 3' 5' 3' 5' 3' 5'  
TTTTTCTCTCATGAAACAACTTACATGAACTTATTTCTCTTCTTCACAGTTGGACATT  
TTTTTCTCTCATGAAACAACTTACATGAACTTATTTCTCTTCTTCACAGTTGGACATT  
TTTTTCTCTCATGAAACAACTTACATGAACTTATTTCTCTTCTTCACAGTTGGACATT  
**Sequence 6**

CTTGGCCCATCTCTTCTGTTCTCAAGATAACTCTTCCTTCACAGTTGGACATT  
3' 5' 3' 5' 3' 5' 3' 5' 3' 5' 3' 5'  
CTTGGCCCATCTCTTCTGTTCTCAAGATAACTCTTCCTTCACAGTTGGACATT  
CTTGGCCCATCTCTTCTGTTCTCAAGATAACTCTTCCTTCACAGTTGGACATT  
CTTGGCCCATCTCTTCTGTTCTCAAGATAACTCTTCCTTCACAGTTGGACATT  
**Sequence 7**

ATACATTTTCAACCTTCTCTTCTGTTCTCAAGATAACTCTTCCTTCACAGTTGGACATT  
4' 3' 2' 1' 5' 4' 3' 2' 1' 5' 4' 3' 2' 1' 5'  
ATACATTTTCAACCTTCTCTTCTGTTCTCAAGATAACTCTTCCTTCACAGTTGGACATT  
ATACATTTTCAACCTTCTCTTCTGTTCTCAAGATAACTCTTCCTTCACAGTTGGACATT  
ATACATTTTCAACCTTCTCTTCTGTTCTCAAGATAACTCTTCCTTCACAGTTGGACATT  
**Sequence 8**

GGGATTTTACACGGTTTACACGGTTTACACGGTTTACACGGTTTACACGGTTTACACGGTT  
4' 3' 2' 1' 5' 4' 3' 2' 1' 5' 4' 3' 2' 1' 5'  
GGGATTTTACACGGTTTACACGGTTTACACGGTTTACACGGTTTACACGGTTTACACGGTT  
GGGATTTTACACGGTTTACACGGTTTACACGGTTTACACGGTTTACACGGTTTACACGGTT  
GGGATTTTACACGGTTTACACGGTTTACACGGTTTACACGGTTTACACGGTTTACACGGTT  
**Sequence 9**

GGGATGATGACAGTTTCGGATGTTTGATGATGACAGTTTCGGATGTTTGATGATGACAG  
4' 3' 2' 1' 5' 4' 3' 2' 1' 5' 4' 3' 2' 1' 5'  
GGGATGATGACAGTTTCGGATGTTTGATGATGACAGTTTCGGATGTTTGATGATGACAG  
GGGATGATGACAGTTTCGGATGTTTGATGATGACAGTTTCGGATGTTTGATGATGACAG  
GGGATGATGACAGTTTCGGATGTTTGATGATGACAGTTTCGGATGTTTGATGATGACAG  
**Sequence 10**

TTCATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT  
4' 3' 2' 1' 5' 4' 3' 2' 1' 5' 4' 3' 2' 1' 5'  
TTCATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT  
TTCATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT  
TTCATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT

FIGURE 4 (CONT.)

**Sequence:**

ATGGGATCACTTGCACGATGAGGTCTTGATGAGGCGAACTTCTTAAATCATTCTTAAACCTTCACAC

4695 4696 4697 4698 4699 4700 4701 4702

ATGGGATCACTTGCACGATGAGGTCTTGATGAGGCGAACTTCTTAAATCATTCTTAAACCTTCACAC 4695

ATGGGATCACTTGCACGATGAGGTCTTGATGAGGCGAACTTCTTAAATCATTCTTAAACCTTCACAC 4696

ATGGGATCACTTGCACGATGAGGTCTTGATGAGGCGAACTTCTTAAATCATTCTTAAACCTTCACAC 4697

**Sequence:**

GGGGACGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG

4703 4704 4705 4706 4707 4708 4709 4710

GGGGACGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4703

GGGGACGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4704

GGGGACGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4705

**Sequence:**

GGGGGATGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG

4711 4712 4713 4714 4715 4716 4717 4718

GGGGGATGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4711

GGGGGATGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4712

GGGGGATGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4713

GGGGGATGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4714

GGGGGATGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4715

**Sequence:**

GGGGGGGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG

4719 4720 4721 4722 4723 4724 4725 4726

GGGGGGGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4719

GGGGGGGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4720

GGGGGGGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4721

GGGGGGGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4722

GGGGGGGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4723

GGGGGGGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4724

GGGGGGGGGATGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4725

**Sequence:**

CAGTCGACGCGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG

4727 4728 4729 4730 4731 4732 4733 4734

CAGTCGACGCGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4727

CAGTCGACGCGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4728

CAGTCGACGCGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGGTTTACATGGGG 4729

FIGURE 4 (CONT.)

1 MYVYMDORSN VNGDSSGARKE EGTDPSOOP RXKGDIRAAI PKHCKWKSPL RMSMSYVARDI  
61 FAVALAMMA VYEDSNTLP LYVVAQCTLE MAIEFLGHDG GIGSESSDPL LNSVYCHIHT  
121 SELUNIING RISERHINON HGVENODESS VELPESERKL ATTSTCWSIM LATLVLISPL VSPVTLKXY  
181 IYLVWRSPOK EGSHENYSS LIPASERKL PWR GRMNSVLRG LTTLDNRYGI TUNNHIDIST  
241 GVPYI ITVWM LDANVYLHIN GHOKLPLWYR GRMNSVLRG LTTLDNRYGI TUNNHIDIST  
301 HYIHHLPOL PHVILVYDTR MAMVHLGKV RPEKTSGAI P THLVESSLWS IKKDHXVSDT  
361 GDIVVETOP DIVVAYSKS KIN.

1

**BRASSICA PLANTS YIELDING OILS WITH A LOW ALPHA LINOLENIC ACID CONTENT****CROSS REFERENCE TO RELATED APPLICATIONS**

This application is a continuation of International Patent Application No. PCT/US2011/037864, filed May 25, 2011, which claims priority under 35 U.S.C. §119(e) to U.S. Provisional Application Ser. No. 61/348,121, filed May 25, 2010.

**TECHNICAL FIELD**

This invention relates to *Brassica* plants, and more particularly, *Brassica* plants having a modified allele at a fatty acid desaturase 3D locus and/or a fatty acid desaturase 3E locus and yielding an oil with a low alpha linolenic acid content in combination with a typical, mid, or high oleic acid content.

**BACKGROUND**

Canola oil contains a relatively high (8%-10%) alpha-linolenic acid (ALA) content. This trienoic fatty acid is unstable and easily oxidized during cooking, which in turn creates off-flavors of the oil. It also develops off odors and rancid flavors during storage (Hawrysh, 1990, Stability of canola oil, Chapter 7, pp. 99-122 In: F. Shahidi, ed. Canola and Rapeseed: Production, Chemistry, Nutrition, and Processing Technology, Van Nostrand Reinhold, N.Y.). Reducing the ALA content level by hydrogenation increases oxidative stability of the oil. However, hydrogenation results in the production of trans fatty acids, which increases the risk for coronary heart disease when consumed. Although an oil's oxidative stability is not determined solely by fatty acid profile, a decrease in the ALA content of canola oils generally improves the stability of the oils.

**SUMMARY**

This document is based on the discovery of a modified fad3D allele and a modified fad3E allele, and use of such alleles in *Brassica* plants to control ALA content. As described herein, *Brassica* plants containing such a modified fad3D allele and modified fad3E allele can produce oils with a low ALA content (i.e. 1.5% or less ALA). Such *Brassica* plants also can include other modified fatty acid desaturase alleles (e.g., fad2 or fad3), fatty acyl-acyl carrier protein thioesterase A2 (fatA2), and/or fatty acyl-acyl carrier protein thioesterase B (fatB) alleles to tailor the oleic acid and total saturated fatty acid content to the desired end use of the oil. *Brassica* plants described herein are particularly useful for producing canola oils for certain food applications as the plants are not genetically modified.

In one aspect, this document features a *Brassica* plant (e.g., *Brassica napus*, *Brassica juncea*, or *Brassica rapa* plant), progeny, and seeds of the plant that include a modified allele at a fatty acid desaturase 3D (fad3D) locus and/or a fatty acid desaturase 3E (fad3E) locus, wherein the modified allele results in the production of a FAD3D and/or FAD3E polypeptide having reduced desaturase activity relative to a corresponding wild-type polypeptide. The fad3E modified allele can include a nucleic acid encoding a truncated FAD3E polypeptide, a nucleic acid encoding a FAD3E polypeptide having a non-conservative substitution of a residue affecting substrate specificity, or a nucleic acid encoding a FAD3E polypeptide having a non-conservative substitution of a residue affecting catalytic activity. In some embodiments, the

2

fad3E modified allele includes a mutation in a splice donor site. A modified fad3E allele can include a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence set forth in SEQ ID NO:1. The fad3D modified allele can include a nucleic acid encoding a truncated FAD3D polypeptide, a nucleic acid having a deletion of an exon or a portion thereof (e.g., a deletion within exon 1 of the nucleic acid). In some embodiments, the fad3D modified allele includes a nucleotide sequence having at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO:32. A plant can include fad3E and fad3D modified alleles. The fad3E and fad3D modified alleles can be mutant alleles. A plant can be an F<sub>1</sub> hybrid.

Any of the plants described herein further can include a modified allele at a fad3A locus and/or a modified allele at a fad3B locus. The fad3A and/or fad3B modified alleles can be mutant alleles. For example, a fad3A modified allele can be selected from the group consisting of a) a nucleic acid encoding a FAD3A polypeptide having a cysteine substituted for arginine at position 275 and b) a nucleic acid encoding a truncated FAD3A polypeptide. A fad3B modified allele can be selected from the group consisting of a) a nucleic acid having a mutation in an exon-intron splice site recognition sequence and b) a nucleic acid encoding a truncated FAD3B polypeptide. Such plants can produce seeds yielding an oil having an ALA content of 0.6 to 1.5%.

Plants described herein can produce seeds yielding an oil having a stearic acid content of 2.5 to 6%.

Any of the plants described herein further can include a modified allele at a delta-12 fatty acid desaturase (fad2) locus. The fad2 modified allele can include a nucleic acid encoding a FAD2 polypeptide having a lysine substituted for glutamic acid in a His-Glu-Cys-Gly-His motif (SEQ ID NO:26). The fad2 modified allele comprising a nucleic acid encoding a FAD2 polypeptide having a glutamic acid substituted for glycine in the DRDYGILNKV motif (SEQ ID NO:28) or a histidine substituted for leucine in a KYLNNP motif (SEQ ID NO:27).

Any of the plants described herein further can include a modified allele at two different fad2 loci. One fad2 modified allele can include a nucleic acid encoding a FAD2 polypeptide having a lysine substituted for glutamic acid in a His-Glu-Cys-Gly-His motif (SEQ ID NO:26). One fad2 modified allele can include a nucleic acid encoding a FAD2 polypeptide having a glutamic acid substituted for glycine in the DRDYGILNKV motif (SEQ ID NO:28) or a histidine substituted for leucine in a KYLNNP motif (SEQ ID NO:27).

Any of the plants described herein further can include a modified allele at a fatty acyl-acyl-ACP thioesterase A2 (fatA2) locus and/or a fatty acyl-acyl-ACP thioesterase B (fatB) locus. The fatA2 and/or fatB modified alleles can be mutant alleles. A fatA2 modified allele results in the production of a FATA2 polypeptide having reduced thioesterase activity relative to a corresponding wild-type FATA2 polypeptide. The fatA2 modified allele can include a nucleic acid encoding a FATA2 polypeptide having a mutation in a region (SEQ ID NO:11) corresponding to amino acids 242 to 277 of the FATA2 polypeptide. The FATA2 polypeptide can include a substitution of a leucine residue for proline at position 255. A fatB modified allele results in the production of a FATB polypeptide having reduced thioesterase activity relative to a corresponding wild-type FATB polypeptide. A plant can include modified alleles at four different fatB loci. At least one of the fatB modified alleles can include a nucleic acid encoding a truncated FATB polypeptide. For example, a truncated FATB polypeptide can include a nucleotide sequence

selected from the group consisting of: SEQ ID NO:22 SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25.

In another aspect, this document features a method of producing an oil. The method includes crushing seeds produced from at least one *Brassica* plant described herein; and extracting oil from the crushed seeds, wherein the oil has, after refining, bleaching, and deodorizing, an ALA content of 0.6 to 1.5%. The oil further can have a stearic acid content of 2.5 to 6.0%.

This document also features a method for making a *Brassica* progeny plant. The method includes crossing one or more first *Brassica* parent plants comprising a modified allele at a fad3E locus and/or a fad3D locus and one or more second *Brassica* parent plants comprising a modified allele at a different fad3 locus, wherein each modified allele results in the production of a FAD3 polypeptide having reduced desaturase activity relative to a corresponding wild-type FAD3 polypeptide; and selecting, for one to five generations, for progeny plants having a modified allele at the fad3E locus and/or fad3D locus, and the modified allele at the different fad3 locus, thereby obtaining the *Brassica* plant.

In another aspect, this document features a method for making a *Brassica* plant. The method includes obtaining one or more first *Brassica* parent plants comprising a modified allele at a fad3E locus and/or modified allele at a fad3D locus, wherein the fad3E or fad3D modified allele results in the production of a FAD3E or FAD3D polypeptide having reduced desaturase activity relative to a corresponding wild-type FAD3 polypeptide; obtaining one or more second *Brassica* parent plants comprising a modified allele at a fad2 locus, the fad2 modified allele comprising a nucleic acid encoding a FAD2 polypeptide having a lysine substituted for glycine in a His-Glu-Cys-Gly-His motif (SEQ ID NO:26); crossing the one or more first *Brassica* parent plants and the one or more second *Brassica* parent plants; and selecting, for one to five generations, for progeny plants having the modified allele at the fad3E locus and/or fad3D locus, and the modified allele at the fad2 locus thereby obtaining the *Brassica* plant. The first *Brassica* parent plant can include a modified allele at three different fad3 loci (e.g., fad3D, fad3A and fad3B).

In another aspect, this document features a method for making a *Brassica* plant. The method includes obtaining one or more first *Brassica* parent plants comprising a modified allele at a fad3E locus and/or fad3D locus, wherein the fad3E or said fad3D modified allele results in the production of a FAD3E or FAD3D polypeptide having reduced desaturase activity relative to a corresponding wild-type FAD3 polypeptide; obtaining one or more second *Brassica* parent plants comprising a modified allele at a fatA2 locus, the fatA2 modified allele comprising a nucleic acid encoding a FATA2 polypeptide having a mutation in a region (SEQ ID NO:11) corresponding to amino acids 242 to 277 of the FADA2 polypeptide; crossing the one or more first *Brassica* parent plants and the one or more second *Brassica* parent plants; and selecting, for one to five generations, for progeny plants having the modified allele at the fad3E locus and/or the fad3D locus, and the modified allele at the fatA2 locus thereby obtaining the *Brassica* plant. The first *Brassica* parent plant further can include a modified allele at a fad2 locus, a modified allele at a fad3A locus, and a modified allele at a fad3B locus, wherein the fad2 modified allele comprising a nucleic acid encoding a FAD2 polypeptide having a lysine substituted for glutamic acid in a His-Glu-Cys-Gly-His motif (SEQ ID N:26), the fad3A modified allele comprising a nucleic acid encoding a FAD3A polypeptide having a cysteine substituted for arginine at position 275, and the fad3B modified allele

comprising a fad3B nucleic acid sequence having a mutation in an exon-intron splice site recognition sequence.

This document also features a method for making a *Brassica* plant. The method includes obtaining one or more first *Brassica* parent plants comprising a modified allele at a fad3E locus or a fad3D locus, wherein the fad3E or fad3D modified allele results in the production of a FAD3E or FAD3D polypeptide having reduced desaturase activity relative to a corresponding wild-type FAD3 polypeptide; obtaining one or more second *Brassica* parent plants comprising at least one modified allele at a fatB locus, wherein the fatB modified allele results in the production of a FATB polypeptide having reduced thioesterase activity relative to a corresponding wild-type FATB polypeptide; crossing the one or more first *Brassica* parent plants and the one or more second *Brassica* parent plants; and selecting, for one to five generations, for progeny plants having the modified allele at the fad3E locus and/or fad3D locus, and the at least one modified fatB allele at the fatB locus, thereby obtaining the *Brassica* plant. The one or more second *Brassica* plants can include modified alleles at four different fatB loci. At least one of the fatB modified alleles can include a nucleic acid encoding a truncated FATB polypeptide.

In another aspect, this document features seeds of a *Brassica* plant comprising a modified allele at a fad3E locus and/or a modified allele at a fad3D locus. The fad3E modified allele can include a nucleic acid having a mutation in a splice donor site. The fad3D modified allele can include a nucleic acid having a deletion of a portion of exon 1. The seeds can yield an oil having an ALA content of 0.6% to 1.5%. The seeds can be F<sub>2</sub> seeds. The *Brassica* plant further can include modified alleles at fad3A and/or fad3B loci. The *Brassica* plant further can include a modified allele at a fad2 locus. The *Brassica* plant further can include a modified allele at a fatB locus. The *Brassica* plant further can include a modified allele at a fatA2 locus. The *Brassica* plant further can include modified alleles at fad3A, fad3B, fad2, fatB, and fatA2 loci.

In yet another aspect, this document features a plant cell of a plant described herein, wherein the plant cell includes one or more of the modified alleles.

This document also features an isolated nucleic acid that includes a nucleic acid sequence selected from the group consisting of i) the nucleic acid sequence set forth in SEQ ID NO:1; ii) the nucleic acid sequence set forth in SEQ ID NO:32; iii) the complement of the nucleic acid sequence set forth in i) or ii); and iv) a nucleic acid fragment of i), ii), or iii) that is at least 50 nucleotides in length and distinguishes a mutant fad3D or fad3E allele from a wild-type fad3D or fad3E allele.

In another aspect, this document features a method of making a plant line. The method includes providing a population of plants; identifying one or more plants in the population containing a modified allele at a fad3E locus and/or a fad3D locus, wherein the modified allele results in the production of a FAD3E or FAD3D polypeptide having reduced desaturase activity relative to a corresponding wild-type FAD3 polypeptide; crossing one or more of the identified plants with itself or a different plant to produce seed; crossing at least one progeny plant grown from the seed with itself or a different plant; and repeating the crossing steps for an additional 0-5 generations to make the plant line, wherein the modified allele at the fad3E locus and/or the fad3D locus is present in the plant line.

This document also features *Brassica napus* seed designated 1904 and represented by American Type Culture Col-

lection (ATCC) Accession No. PTA-11273, as well as progeny of the seed designated 1904 and represented by ATCC Accession No. PTA-11273.

This document also features *Brassica napus* seed designated 2558 and represented by American Type Culture Collection (ATCC) Accession No. PTA-11274, as well as progeny of the seed designated 2558 and represented by ATCC Accession No. PTA-11274.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All numbers expressing quantities of ingredients, properties such as molecular weight, percentages, reaction conditions, and so forth used in the specification and claims are to be understood as being modified by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth are approximations that may depend upon the desired properties sought.

Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

#### DESCRIPTION OF DRAWINGS

FIG. 1 is an alignment of the BnFad3E sequences from 1904, IMC201, and BrFad3E (SEQ ID NOs:1, 2, and 3, respectively). The BnFad3E-2 SNP that correlates with the low ALA (C18:3) content in the 1904 mutant line is highlighted with a black box at position 1851 of this alignment. At the position 1756 in SEQ ID NO:1 (1904 BnFad3E-2.seq), a single nucleotide mutation (G to A) is shown, which is located in a splice donor site (see FIG. 2). The start codon (ATG) is underlined at position 94 of this sequence alignment and the stop codon in BrFad3E (TAA) is at position 3828.

FIG. 2 is an alignment of the nucleotide sequence of the exon 3, intron 3 border of the BnFad3A, BnFad3B, BnFad3E genes from IMC201, IMC02, Westar, 1904, 2558, and 95CB504, and the BrFad3E gene from *Brassica rapa* (world wide web at [brassica-rapa.org](http://brassica-rapa.org)) showing the single nucleotide mutation (G to A) in BnFad3E-2 from the 1904 line. See SEQ ID NOs:4-8. This mutation (G to A) is located in the last nucleotide of exon 3 of BnFad3E. Intron 3 of the BnFad3E starts from the sequence GT (see SEQ ID NO:8).

FIG. 3 is an alignment of the amino acid sequences of FAD3E polypeptides from *B. napus* and *B. rapa*, and FAD3 from *Arabidopsis thaliana*. See SEQ ID NOs:29, 30, and 31.

FIG. 4 is an alignment of the BnFad3D sequences from 1904 (SEQ ID NO:32) and IMC201 and 95CB504 (SEQ ID NO:33) showing a DNA deletion in the 1904 BnFad3D starting at position 575 in this alignment, which includes a portion of exon 1 and intron 1. The start codon (ATG) is at position 441.

FIG. 5 is the amino acid sequence of the FAD3D polypeptide (SEQ ID NO:34). In line 1904, the FAD3D polypeptide is truncated after amino acid 64.

Like reference symbols in the various drawings indicate like elements.

#### DETAILED DESCRIPTION

In general, this document provides *Brassica* plants, including *B. napus*, *B. juncea*, and *B. rapa* species of *Brassica*, that yield seeds producing oils having a low ALA content (i.e., 1.6% or less). Canola oil produced from seeds having a low ALA content tends to exhibit increased stability (e.g., oxidative stability and/or flavor stability) and a useful nutritional profile, and can be used for many food applications including as a frying oil.

In some embodiments, plants described herein yield seeds producing oils having a low ALA content in combination with low total saturated fatty acids (i.e., 6% or less) or very low total saturated fatty acids (i.e., having 3.6% or less). As used herein, total saturated fatty acid content refers to the total of myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0), and lignoceric acid (C24:0). For example, *Brassica* plants described herein can produce oils having a low ALA content and a total saturated fatty acid content of 2.5 to 6.0%, 3 to 5%, 3 to 4.5%, 3.25 to 3.75%, 3.0 to 3.5%, 3.4 to 3.7%, 3.6 to 5%, 4 to 5.5%, 4 to 5%, or 4.25 to 5.25%. Oils having a low ALA content and a low or very low total saturated fatty acid content have improved oxidative stability and nutritional quality and can help consumers reduce their intake of saturated fatty acids.

In some embodiments, *Brassica* plants yield seed oils having a low ALA content in combination with a typical (60%-70%), mid (70.1%-80%), or high (>80%) oleic acid content. In some embodiments, the total saturated fatty acid content of such seed oils can be less than 6%. As such, *Brassica* plants can produce seed oils having a fatty acid content tailored to the desired end use of the oil (e.g., frying or food applications). For example, *Brassica* plants can be produced that yield seeds having a low ALA content (e.g., 1.5% or less), an oleic acid content of 60 to 70%, and a linoleic acid content of 17 to 24%. Canola oils having such a fatty acid profile are particularly useful for frying applications due to the polyunsaturated fatty acid content, which is low enough to have improved oxidative stability for frying yet high enough to impart the desired fried flavor to the food being fried, and are an improvement over commodity type canola oils. The fatty acid content of commodity type canola oils may be on the order of 6 to 8% total saturated fatty acids, 55 to 65% oleic acid, 20 to 30% linoleic acid, and 7 to 10%  $\alpha$ -linolenic acid. See, e.g., *Bailey's Industrial Oil and Fat Products*, Section 2.2, "Canola Oil" on pages 61-121 of Volume 2 (6th Edition, 2005).

In some embodiments, *Brassica* plants can be produced that yield seeds having a low ALA content, mid-oleic acid content (e.g., 70.1 to 80% oleic acid) and a low total saturated fatty acid content (e.g., <6.0%). Canola oils having such a fatty acid profile have an oxidative stability that is higher than oils with higher ALA and lower oleic acid contents or commodity type canola oils, and are useful for coating applications (e.g., spray-coatings), formulating food products, or other applications where shelf-life stability is desired. In addition, *Brassica* plants can be produced that yield seeds having a low ALA content, high oleic acid content (e.g., 80.1 to 90% oleic acid) and a low total saturated fatty acid content (<6.0%). Canola oils having a low ALA, high oleic acid, and low total saturated fatty acid content are particularly useful for food applications requiring high oxidative stability and a reduced saturated fatty acid content.

*Brassica* Plants

*Brassica* plants described herein can have reduced levels of ALA (e.g., 8.0% or less) in the seed oil as a result of reduced activity of fatty acid desaturase (FAD) 3E (also known as delta-15 desaturase). *Brassica* plants described herein also can have reduced levels of ALA (e.g., 3.0% or less, 2.8% or less, 2.6% or less) in the seed oil as a result of reduced activity of FAD3D. FAD3 proteins are involved in the enzymatic conversion of linoleic acid to α-linolenic acid. Sequences of higher plant Fad3 genes are disclosed in Yadav et al., *Plant Physiol.*, 103:467-476 (1993), WO 93/11245, and Arondel et al., *Science*, 258:1353-1355 (1992). It is understood that throughout the disclosure, reference to “plant” or “plants” includes progeny, i.e., descendants of a particular plant or plant line, as well as cells or tissues from the plant. Progeny of an instant plant include seeds formed on F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub> and subsequent generation plants, or seeds formed on BC<sub>1</sub>, BC<sub>2</sub>, BC<sub>3</sub>, and subsequent generation plants. Seeds produced by a plant can be grown and then selfed (or outcrossed and selfed, or doubled through dihaploid) to obtain seeds homozygous for a modified allele. The term “allele” or “alleles” refers to one or more alternative forms of a gene at a particular locus. As used herein, a “line” is a group of plants that display little or no genetic variation between individuals for at least one trait. Such lines may be created by several generations of self-pollination and selection, or vegetative propagation from a single parent using tissue or cell culture techniques. As used herein, the term “variety” refers to a line which is used for commercial production, and includes hybrid varieties and open-pollinated varieties.

Reduced activity, including absence of detectable desaturase activity, of FAD3E and/or FAD3D can be achieved by modifying an endogenous fad3E or fad3D allele. An endogenous fad3E or fad3D allele can be modified by, for example, mutagenesis or by using homologous recombination to replace an endogenous plant gene with a variant containing one or more mutations (e.g., produced using site-directed mutagenesis). See, e.g., Townsend et al., *Nature* 459:442-445 (2009); Tovkach et al., *Plant J.*, 57:747-757 (2009); and Lloyd et al., *Proc. Natl. Acad. Sci. USA*, 102:2232-2237 (2005). Similarly, for other genes discussed herein, the endogenous allele can be modified by mutagenesis or by using homologous recombination to replace an endogenous gene with a variant. Modified alleles obtained through mutagenesis are encompassed by the term “mutant alleles” as that term is used herein.

Reduced desaturase activity, including absence of detectable activity, can be inferred from the decreased level of linolenic acid (product) and in some cases, increased level of linoleic acid (the substrate) in the plant compared with a corresponding control plant. Reduced activity also can be assessed by in vitro translation of the desaturase and assaying for desaturase activity. See, for example, Goren and Fox, *Protein Expr Purif.* 62(2): 171-178 (2008).

Genetic mutations can be introduced within a population of seeds or regenerable plant tissue using one or more mutagenic agents. Suitable mutagenic agents include, for example, ethyl methane sulfonate (EMS), methyl N-nitrosoguanidine (MNNG), ethidium bromide, diepoxybutane, ionizing radiation, x-rays, UV rays and other mutagens known in the art. In some embodiments, a combination of mutagens, such as EMS and MNNG, can be used to induce mutagenesis. The treated population, or a subsequent generation of that population, can be screened for reduced desaturase activity that results from the mutation, e.g., by determining the fatty acid profile of the population and comparing it to that of a corresponding non-mutagenized population. Mutations can be in

any portion of a gene, including coding sequence, exon sequence, intron sequence, and regulatory elements, that render the resulting gene product non-functional or with reduced activity. Suitable types of mutations include, for example, 5 insertions or deletions of nucleotides, and transitions or transversions in the wild-type coding sequence. Such mutations can lead to deletion or insertion of amino acids, and conservative or non-conservative amino acid substitutions in the corresponding gene product. In some embodiments, the 10 mutation is a deletion of an exon or a portion thereof, resulting in the production of a truncated polypeptide from either lack of or incorrect RNA splicing. In some embodiments, the mutation is a nonsense mutation, which results in the introduction of a stop codon (TGA, TAA, or TAG) and production 15 of a truncated polypeptide. The gene product of an allele having a stop codon mutation typically lacks detectable desaturase activity. In some embodiments, the mutation is a splice site mutation which alters or abolishes the correct splicing of the pre-mRNA sequence, resulting in a protein of 20 different amino acid sequence than the wild type. For example, one or more exons may be skipped during RNA splicing, resulting in a protein lacking the amino acids encoded by the skipped exons. Alternatively, the reading frame may be altered by incorrect splicing, one or more 25 introns may be retained, alternate splice donors or acceptors may be generated, or splicing may be initiated at an alternate position, or alternative polyadenylation signals may be generated. In some embodiments, more than one mutation or 30 more than one type of mutation is introduced. PCR can be used to amplify modified alleles in genomic DNA from the plant or plant tissue, and the resulting amplification product can be isolated and sequenced to characterize the polypeptide encoded by the modified allele. In some embodiments, RT-PCR can be used to detect particular RNA transcripts.

35 Insertions, deletions, or substitutions of amino acids in a protein sequence may, for example, disrupt the conformation of essential alpha-helical or beta-pleated sheet regions of the resulting gene product. Amino acid insertions, deletions, or substitutions also can disrupt binding, alter substrate specificity, or disrupt catalytic sites important for gene product 40 activity. It is known in the art that the insertion or deletion of a larger number of contiguous amino acids is more likely to render the gene product non-functional, compared to a smaller number of inserted or deleted amino acids. Non-conservative amino acid substitutions may replace an amino acid of one class with an amino acid of a different class. Non-conservative substitutions may make a substantial 45 change in the charge or hydrophobicity of the gene product. Non-conservative amino acid substitutions may also make a substantial change in the bulk of the residue side chain, e.g., substituting an alanine residue for an isoleucine residue.

Examples of non-conservative substitutions include the substitution of a basic amino acid for a non-polar amino acid, or a polar amino acid for an acidic amino acid. Because there 50 are only 20 amino acids encoded in a gene, substitutions that result in reduced activity may be determined by routine experimentation, incorporating amino acids of a different class in the region of the gene product targeted for mutation.

In some embodiments, a plant described herein contains a 55 modified allele at a fad3E locus. For example, a fad3E locus can include a nucleotide sequence having at least 90% (e.g., at least 91, 92, 93, 94, 95, 96, 97, 98, or 99%) sequence identity to the nucleotide sequence set forth in SEQ ID NO:1. The nucleotide sequence set forth in SEQ ID NO:1 is a representative 60 nucleotide sequence of the fad3E gene from *B. napus* line 1904, which contains a single nucleotide mutation (G to A) in a splice donor site. As used herein, the term “sequence

“identity” refers to the degree of similarity between any given nucleic acid sequence and a target nucleic acid sequence. The degree of similarity is represented as percent sequence identity. Percent sequence identity is calculated by determining the number of matched positions in aligned nucleic acid sequences, dividing the number of matched positions by the total number of aligned nucleotides, and multiplying by 100. A matched position refers to a position in which identical nucleotides occur at the same position in aligned nucleic acid sequences. Percent sequence identity also can be determined for any amino acid sequence. Percent sequence identity can be determined using the BLAST 2 Sequences (Bl2seq) program from the stand-alone version of BLASTZ containing BLASTN version 2.0.14 and BLASTP version 2.0.14. This stand-alone version of BLASTZ can be obtained from Fish & Richardson’s web site (World Wide Web at “fr” dot “com” slash “blast”) or the U.S. government’s National Center for Biotechnology Information web site (World Wide Web at “ncbi” dot “nlm” dot “nih” dot “gov”). Instructions explaining how to use the Bl2seq program can be found in the readme file accompanying BLASTZ.

Bl2seq performs a comparison between two sequences using either the BLASTN or BLASTP algorithm. BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. To compare two nucleic acid sequences, the options are set as follows: -i is set to a file containing the first nucleic acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second nucleic acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastn; -o is set to any desired file name (e.g., C:\output.txt); -q is set to -1; -r is set to 2; and all other options are left at their default setting. The following command will generate an output file containing a comparison between two sequences: C:\Bl2seq -i c:\seq1.txt -j c:\seq2.txt -p blastn -o c:\output.txt -q -1 -r 2. If the target sequence shares homology with any portion of the identified sequence, then the designated output file will present those regions of homology as aligned sequences. If the target sequence does not share homology with any portion of the identified sequence, then the designated output file will not present aligned sequences.

Once aligned, a length is determined by counting the number of consecutive nucleotides from the target sequence presented in alignment with sequence from the identified sequence starting with any matched position and ending with any other matched position. A matched position is any position where an identical nucleotide is presented in both the target and identified sequence. Gaps presented in the target sequence are not counted since gaps are not nucleotides. Likewise, gaps presented in the identified sequence are not counted since target sequence nucleotides are counted, not nucleotides from the identified sequence.

The percent identity over a particular length is determined by counting the number of matched positions over that length and dividing that number by the length followed by multiplying the resulting value by 100. For example, if (i) a 500-base nucleic acid target sequence is compared to a subject nucleic acid sequence, (ii) the Bl2seq program presents 200 bases from the target sequence aligned with a region of the subject sequence where the first and last bases of that 200-base region are matches, and (iii) the number of matches over those 200 aligned bases is 180, then the 500-base nucleic acid target sequence contains a length of 200 and a sequence identity over that length of 90% (i.e., 180, 200×100=90).

It will be appreciated that different regions within a single nucleic acid target sequence that aligns with an identified sequence can each have their own percent identity. It is noted that the percent identity value is rounded to the nearest tenth.

For example, 78.11, 78.12, 78.13, and 78.14 are rounded down to 78.1, while 78.15, 78.16, 78.17, 78.18, and 78.19 are rounded up to 78.2. It also is noted that the length value will always be an integer.

In some embodiments, a plant described herein contains a modified allele at a fad3D locus. For example, a fad3D locus can include a nucleotide sequence having at least 90% (e.g., at least 91, 92, 93, 94, 95, 96, 97, 98, or 99%) sequence identity to the nucleotide sequence set forth in SEQ ID NO:32. The nucleotide sequence set forth in SEQ ID NO:32 is a representative nucleotide sequence of the fad3D gene from *B. napus* line 1904, which contains a deletion of 164 nucleotides from exon 1. In *B. napus* line IMC201 and 95CB504, exon 1 starts at position 441 and ends at position 739. See, e.g., FIG. 4.

In some embodiments, a *Brassica* plant contains a modified fad3E allele and a modified fad3D allele. A modified fad3E and a modified fad3D allele may be combined in a plant by making a genetic cross between modified lines. For example, a plant having a modified allele at a fad3E locus can be crossed or mated with a second plant having a modified allele at a fad3D locus. Seeds produced from the cross are planted and the resulting plants are selfed in order to obtain progeny seeds. These progeny seeds can be screened in order to identify those seeds carrying both modified alleles. In some embodiments, progeny are selected over multiple generations (e.g., 2 to 5 generations) to obtain plants having modified fad3E and fad3D alleles. In some embodiments, a line having both fad3E and fad3D modified alleles is used to introgress an individual modified allele into a different line or to introgress both modified alleles into a different line.

In some embodiments, a *Brassica* plant contains a modified fad3E allele or a modified fad3D allele, and optionally one or more modified alleles at fad3 (e.g. fad3A and/or fad3B), fatA2, fatB, and fad2 loci. In some embodiments, a *Brassica* plant contains a modified fad3E allele and a modified fad3D allele, and optionally one or more modified alleles at fad3 (e.g., fad3A and/or fad3B), fatA2, fatB, and fad2 loci. For example, a *Brassica* plant can contain a modified fad3E allele, a modified fad3D allele, and one or more other modified fad3 alleles. For example, in addition to a modified fad3E and fad3D allele, *Brassica* plants can contain the mutation from the APOLLO or STELLAR *B. napus* variety that confers low linolenic acid. The STELLAR and APOLLO varieties were developed at the University of Manitoba (Manitoba, Canada). In some embodiments, the disclosed plants contain the fad3A and/or fad3B mutation from IMC02 that confer a low linolenic acid phenotype. IMC02 contains a mutation in both the fad3A and fad3B genes. The fad3A gene contains a C to T mutation at position 2565, numbered from the ATG in genomic DNA, resulting in the substitution of a cysteine for arginine at position 275 of the encoded FAD3A polypeptide. The fad3B gene contains a G to A mutation at position 3053 from ATG in genomic DNA, located in the exon-intron splice site recognition sequence. IMC02 was obtained from a cross of IMC01 x Westar. See Example 3 of U.S. Pat. No. 5,750,827. IMC01 was deposited with the American Type Culture Collection (ATCC) under Accession No. 40579. IMC02 was deposited with the ATCC under Accession No. PTA-6221. Other examples of fad3 mutations include nonsense mutations in fad3A and fad3B sequences. See, Example 4. For example, the mutant fad3A sequence set forth in SEQ ID NO:9 contains a mutation at position 102, resulting in a codon change from TGG to TGA and production of a truncated FAD3A polypeptide. The mutant fad3B sequence set forth in SEQ ID NO:10 contains a mutation at position 206, resulting

## 11

in a codon change from TGG to TAG and production of a truncated FAD3B polypeptide.

Two or more different modified fad3 alleles may be combined in a plant by making a genetic cross between modified lines. For example, a plant having a modified allele at a fad3E locus and/or a fad3D locus can be crossed or mated with a second plant having a modified allele at a fad3A or fad3B locus. Seeds produced from the cross are planted and the resulting plants are selfed in order to obtain progeny seeds. These progeny seeds can be screened in order to identify those seeds carrying both modified alleles. In some embodiments, progeny are selected over multiple generations (e.g., 2 to 5 generations) to obtain plants having modified alleles at two different fad3 loci.

*Brassica* plants having a modified allele at a fad3E locus or fad3D locus also can include modified alleles controlling fatty acyl-ACP thioesterase A2 (fatA2) and/or fatty acyl-ACP thioesterase B (fatB) to tailor the total saturated fatty acid content to the end use of the oil. Fatty acyl-ACP thioesterases hydrolyze acyl-ACPs in the chloroplast to release the newly synthesized fatty acid from ACP, effectively removing it from further chain elongation in the plastid. The free fatty acid can then leave the plastid, become bound to Coenzyme A (CoA) and enter the Kennedy pathway in the endoplasmic reticulum (ER) for triacylglycerol (TAG) biosynthesis. Members of the FATA family prefer oleoyl (C18:1) ACP substrates with minor activity towards 18:0 and 16:0-ACPs, while members of the FATB family hydrolyze primarily saturated acyl-ACPs between 8 and 18 carbons in length. See Jones et al., *Plant Cell* 7:359-371 (1995); Ginalski and Rhchlewski, *Nucleic Acids Res* 31:3291-3292 (2003); and Voelker T in Genetic Engineering (Setlow, J K, ed) Vol 18, 111-133, Plenum Publishing Corp., New York (2003).

Reduced activity of FATA2 and/or FATB, including absence of detectable activity, can be inferred from the decreased level of saturated fatty acids in the seed oil compared with seed oil from a corresponding control plant. Reduced activity also can be assessed in plant extracts using assays for fatty acyl-ACP hydrolysis. See, for example, Bonaventure et al., *Plant Cell* 15:1020-1033 (2003); and Eccleston and Ohlrogge, *Plant Cell* 10:613-622 (1998).

In some embodiments, in addition to a modified allele at a fad3E locus and/or a fad3D locus, and optionally one or more other modified fad3 loci, a *Brassica* plant contains a modified allele at a fatA2 locus, wherein the modified allele results in the production of a FATA2 polypeptide having reduced thioesterase activity relative to a corresponding wild-type FATA2 polypeptide. For example, the modified fatA2 allele can include a nucleic acid that encodes a FATA2 polypeptide having a non-conservative substitution within a helix/4-stranded sheet (4HBT) domain (also referred to as a hot-dog domain) or non-conservative substitution of a residue affecting catalytic activity or substrate specificity. For example, a *Brassica* plant can contain a modified allele that includes a nucleic acid encoding a FATA2b polypeptide having a substitution in a region (SEQ ID NO:11) of the polypeptide corresponding to residues 242 to 277 of the FATA2 polypeptide (as numbered based on the alignment to the *Arabidopsis thaliana* FATA2 polypeptide set forth in GenBank Accession No. NP\_193041.1, protein (SEQ ID NO:12); GenBank Accession No. NM\_117374, mRNA). This region of FATA2 is highly conserved in *Arabidopsis* and *Brassica*. In addition, many residues in this region are conserved between FATA and FATB, including the aspartic acid at position 259, asparagine at position 263, histidine at position 265, valine at position 266, asparagine at position 268, and tyrosine at position 271 (as numbered based on the alignment to SEQ ID NO:12). The

## 12

asparagine at position 263 and histidine at position 265 are part of the catalytic triad, and the arginine at position 256 is involved in determining substrate specificity. See also Mayer and Shanklin, *BMC Plant Biology* 7:1-11 (2007). SEQ ID NO:13 sets forth the predicted amino acid sequence of the *Brassica* FATA2b polypeptide encoded by exons 2-6, and corresponding to residues 121 to 343 of the *A. thaliana* sequence set forth in SEQ ID NO:12. For example, the FATA2 polypeptide can have a substitution of a leucine residue for proline at the position corresponding to position 255 of the *Arabidopsis* FATA2 polypeptide (i.e., position 14 of SEQ ID NO:11 or position 135 of SEQ ID NO:13). The proline in the *B. napus* sequence corresponding to position 255 in *Arabidopsis* is conserved among *B. napus*, *B. rapa*, *B. juncea*, *Zea mays*, *Sorghum bicolor*, *Oryza sativa* Indica (rice), *Triticum aestivum*, *Glycine max*, *Jatropha* (tree species), *Carthamus tinctorius*, *Cuphea hookeriana*, *Iris tectorum*, *Perilla frutescens*, *Helianthus annuus*, *Garcinia mangostana*, *Picea sitchensis*, *Physcomitrella patens* subsp. Patens, *Elaeis guineensis*, *mitis vinifera*, *Elaeis oleifera*, *Camellia oleifera*, *Arachis hypogaea*, *Capsicum annuum*, *Cuphea hookeriana*, *Populus trichocarpa*, and *Diploknema butyracea*. The mutation at position 255 is associated with a low total saturated fatty acid phenotype, low stearic acid phenotype, low arachidic acid phenotype, and an increased eicosenoic acid phenotype. The stearic acid content phenotype is negatively correlated with the eicosenoic acid phenotype. See, U.S. Provisional Application Nos. 61/287,985 and 61/295,049.

In some embodiments, the modified allele at a fatA2 locus includes a nucleotide sequence having at least 90% (e.g., at least 91, 92, 93, 94, 95, 96, 97, 98, or 99%) sequence identity to the nucleotide sequence set forth in SEQ ID NO:14 or SEQ ID NO:15. The nucleotide sequences set forth in SEQ ID NOS:14 and 15 are representative nucleotide sequences from the mutant fatA2b gene from *B. napus* line 15.24. See, U.S. Provisional Application Nos. 61/287,985 and 61/295,049.

In some embodiments, a *Brassica* plant contains a modified allele at a fatB locus, wherein the modified allele results in the production of a FATB polypeptide having reduced thioesterase activity relative to a corresponding wild-type FATB polypeptide. In some embodiments, a *Brassica* plant contains modified alleles at two or more different fatB loci. In some embodiments, a *Brassica* plant contains modified alleles at three different fatB loci or contains modified alleles at four different fatB loci. *Brassica* napus contains 6 different FATB isoforms (i.e., different forms of the FATB polypeptide at different loci), which are called isoforms 1-6 herein. SEQ ID NOS:16-21 set forth the nucleotide sequences encoding FATB isoforms 1-6, respectively, of *Brassica* napus. The nucleotide sequences set forth in SEQ ID NOS:16-21 have 82% to 95% sequence identity as measured by the ClustalW algorithm (version 1.83, default parameters). See Chenna et al., *Nucleic Acids Res.*, 31(13):3497-500 (2003).

For example, in addition to a modified allele at a fad3E locus and a fad3D locus, a *Brassica* plant can have a mutation in a nucleotide sequence encoding FATB isoform 1, isoform 2, isoform 3, isoform 4, isoform 5, or isoform 6. In some embodiments, a plant can have a mutation in a nucleotide sequence encoding FAD3E and can have mutation in a nucleotide sequence encoding 2 or more FATB isoforms, e.g., FATB isoforms 1 and 2; 1 and 3; 1 and 4; 1 and 5; 1 and 6; 2 and 3; 2 and 4; 2 and 5; 2 and 6; 3 and 4; 3 and 5; 3 and 6; 4 and 5; 4 and 6; 5 and 6; 1, 2, and 3; 1, 2, and 4; 1, 2, and 5; 1, 2, and 6; 1, 3, and 4; 1, 3, and 5; 1, 3, and 6; 1, 4, and 5; 1, 4, and 6; 1, 5, and 6; 2, 3, and 4; 2, 3, and 5; 2, 3, and 6; 2, 4, and 5; 2, 4, and 6; 1, 5, and 6; 3, 4, and 5; 3, 4, and 6; 3, 5, and 6; 4, 5, and 6; 1, 2, 3, and 4; 1, 2, 3, and 5; 1, 2, 3, and 6; 1, 2, 4,

and 5; 1, 2, 4, and 6; 1, 2, 5, and 6; 1, 3, 4 and 5; 1, 3, 4, and 6; 1, 3, 5, and 6; 1, 4, 5, and 6; 2, 3, 4, and 5; 2, 3, 4 and 6; 2, 3, 5, and 6; 2, 4, 5, and 6; or 3, 4, 5, and 6. In some embodiments, a *Brassica* plant can have a mutation in a nucleotide sequence encoding a FAD3E polypeptide and can have a mutation in nucleotide sequences encoding FATB isoforms 1, 2, and 3; 1, 2, and 4; 1, 3, and 4; 2, 3, and 4; or 1, 2, 3, and 4. In some embodiments, a mutation in a FATB isoform results in deletion of a 4HBT domain or a portion thereof of a FATB polypeptide. FATB polypeptides typically contain a tandem repeat of the 4HBT domain, where the N-terminal 4HBT domain contains residues affecting substrate specificity (e.g., two conserved methionines, a conserved lysine, a conserved valine, and a conserved serine) and the C-terminal 4HBT domain contains residues affecting catalytic activity (e.g., a catalytic triad of a conserved asparagine, a conserved histidine, and a conserved cysteine) and substrate specificity (e.g., a conserved tryptophan). See Mayer and Shanklin, *J. Biol. Chem.* 280:3621-3627 (2005). In some embodiments, the mutation in a nucleotide sequence encoding FATB results in a non-conservative substitution of a residue in a 4HBT domain or a residue affecting substrate specificity. In some embodiments, the mutation in a nucleotide sequence encoding FATB is a splice site mutation. In some embodiment, the mutation in a nucleotide sequence encoding FATB is a non-sense mutation in which a premature stop codon (TGA, TAA, or TAG) is introduced, resulting in the production of a truncated polypeptide.

SEQ ID NOs:22-25 set forth the nucleotide sequences encoding fatB isoforms 1-4, respectively, and containing exemplary nonsense mutations that result in truncated FATB polypeptides. SEQ ID NO:22 is the nucleotide sequence of isoform 1 having a mutation at position 154, which changes the codon from CAG to TAG. SEQ ID NO:23 is the nucleotide sequence of isoform 2 having a mutation at position 695, which changes the codon from CAG to TAG. SEQ ID NO:24 is the nucleotide sequence of isoform 3 having a mutation at position 276, which changes the codon from TGG to TGA. SEQ ID NO:25 is the nucleotide sequence of isoform 4 having a mutation at position 336, which changes the codon from TGG to TGA. See also U.S. Provisional Application Nos. 61/287,985 and 61/295,049.

Two or more different modified FATB alleles may be combined in a plant by making a genetic cross between modified lines. For example, a plant having a modified allele at a FATB locus encoding isoform 1 can be crossed or mated with a second plant having a modified allele at a FATB locus encoding isoform 2. Seeds produced from the cross are planted and the resulting plants are selfed in order to obtain progeny seeds. These progeny seeds can be screened in order to identify those seeds carrying both modified alleles. In some embodiments, progeny are selected over multiple generations (e.g., 2 to 5 generations) to obtain plants having modified alleles at two different FATB loci. Similarly, a plant having modified alleles at two or more different FATB isoforms can be crossed with a second plant having modified alleles at two or more different FATB alleles, and progeny seeds can be screened to identify those seeds carrying modified alleles at four or more different FATB loci. Again, progeny can be selected for multiple generations to obtain the desired plant.

In some embodiments, a modified allele at a fad3E locus, a fad3D locus, a fatA2 locus and modified alleles at two or more (e.g., three or four) different fatB loci can be combined in a plant. For example, a plant having a modified allele at a fad3E locus and a fad3D locus can be crossed or mated with a second plant having a modified allele at a fatA2 locus. Seeds produced from the cross are planted and the resulting plants are

selfed in order to obtain progeny seeds. These progeny seeds can be screened in order to identify those seeds carrying modified fad3E, fad3D, and fatA2 alleles. Progeny can be selected over multiple generations (e.g., 2 to 5 generations) to obtain plants having a modified allele at a fad3E locus, a modified allele at a fad3D locus, and a modified allele at a fatA2 locus. Furthermore, progeny identified as having a modified allele at a fad3E locus, a modified allele at a fad3D locus, and a modified allele at a fatA2 locus can be crossed or mated with a second plant having modified alleles at two or more different fatB loci. Seeds produced from the cross are planted and the resulting plants are selfed in order to obtain progeny seeds. These progeny seeds can be screened in order to identify those seeds carrying modified fad3E, fad3D, fatA2, and fatB alleles. Progeny can be selected over multiple generations (e.g., 2 to 5 generations) to obtain plants having a modified allele at a fad3E locus, a modified allele at a fatA2 locus, and two or more different fatB loci. Plants having a modified allele at a fad3E locus, a fad3D locus, a fatA2b locus, and modified alleles at three or four different fatB loci have a low ALA content, high oleic acid content, and a low total saturated fatty acid content.

*Brassica* plants described herein also can have decreased activity of a delta-12 desaturase, which is involved in the enzymatic conversion of oleic acid to linoleic acid, to confer a mid or high oleic acid content in the seed oil. *Brassica* plants can exhibit reduced activity of delta-12 desaturase (also known as FAD2) in combination with reduced activity of FAD3E and optionally one or more of FAD3A, FAD3B, FATA2, and FATB. The sequences for the wild-type fad2 genes from *B. napus* (termed the D form and the F form) are disclosed in WO 98/56239. A reduction in delta-12 desaturase activity, including absence of detectable activity, can be achieved by mutagenesis. Decreased delta-12 desaturase activity can be inferred from the decrease level of linoleic acid (product) and increased level of oleic acid (substrate) in the plant compared with a corresponding control plant. Non-limiting examples of suitable fad2 mutations include the G to A mutation at nucleotide 316 within the fad2-D gene, which results in the substitution of a lysine residue for glutamic acid in a HECGH (SEQ ID NO:26) motif. Such a mutation is found within the line IMC129, which has been deposited with the ATCC under Accession No. 40811. Another suitable fad2 mutation can be the T to A mutation at nucleotide 515 of the fad2-F gene, which results in the substitution of a histidine residue for leucine in a KYLNNP (SEQ ID NO:27) motif (amino acid 172 of the Fad2 F polypeptide). Such a mutation is found within the variety Q508. See U.S. Pat. No. 6,342,658. Another example of a fad2 mutation is the G to A mutation at nucleotide 908 of the fad2-F gene, which results in the substitution of a glutamic acid for glycine in the DRDYGILNKV (SEQ ID NO:28) motif (amino acid 303 of the Fad2 F polypeptide). Such a mutation is found within the line Q4275, which has been deposited with the ATCC under Accession No. 97569. See U.S. Pat. No. 6,342,658. Another example of a suitable fad2 mutation can be the C to T mutation at nucleotide 1001 of the fad2-F gene (as numbered from the ATG), which results in the substitution of an isoleucine for threonine (amino acid 334 of the Fad2 F polypeptide). Such a mutation is found within the high oleic acid line Q7415.

Typically, the presence of one of the fad2-D or fad2-F mutations confers a mid-oleic acid phenotype (e.g., 70-80% oleic acid) to the seed oil, while the presence of both fad2-D and fad2-F mutations confers a higher oleic acid phenotype (e.g., >80% oleic acid). For example, Q4275 contains the fad2-D mutation from IMC129 and a fad2-F mutation at amino acid 303. Q508 contains fad2-D mutation from

15

IMC129 and a fad2-F mutation at amino acid 172. Q7415 contains the fad2-D mutation from IMC129 and a fad2-F mutation at amino acid 334. The presence of both fad2 mutations in Q4275, Q508, and Q7415 confers a high oleic acid phenotype of greater than 80% oleic acid.

Thus, in some embodiments, a *Brassica* plant contains a modified allele at a fad3E locus and a modified allele at a fad2 locus. For example, a *Brassica* plant can contain a modified allele at a fad3E locus and a modified allele at a fad2 locus described above. A *Brassica* plant also can contain a modified allele at a fad3E locus, a modified allele at a fad2 locus, and a modified allele at a fatA2 locus. A *Brassica* plant can contain a modified allele at a fad3E locus, modified alleles at two or more different fatB loci (three or four different loci), and a fad2 locus described above. A *Brassica* plant also can contain a modified allele at a fad3E locus, fatA2 locus, modified alleles at two or more different fatB loci (three or four different loci) and a modified allele at a fad2 locus described above. In some embodiments, a *Brassica* plant contains a modified allele at a fad3E locus, at least one modified allele at a different fad3 locus, a modified allele at a fatA2 locus, a modified allele at one or more different fatB loci (e.g., two or more), and a modified allele at one or more fad2 loci. A *Brassica* plant also can contain modified alleles at a fad3E locus and a fad3D locus, modified alleles at two or more different fatB loci (three or four different loci), modified alleles at fad2 loci, and modified alleles at fad3A and/or fad3B loci described above. A *Brassica* plant also contains a modified allele at a fad3E locus, a modified allele at a fad3D locus, a modified allele at a fatA2 locus, modified alleles at two or more different FATB loci (three or four different loci), modified alleles at fad2 loci, and modified alleles at fad3A and fad3B loci described above.

One commercially important *Brassica* crop is *B. napus*. Commercial *B. napus* lines may be classified as either spring lines or winter lines. Winter lines are commonly planted in the autumn and flower in the spring after a period of vernalization over the winter. Spring lines do not require vernalization to flower and are commonly planted and harvested in the same growing season. Winter lines are common in Europe, but most winter lines fare poorly in the colder winters of Canada and the northern United States. As a consequence, most *B. napus* grown commercially in North America are spring lines. One useful embodiment provides a *Brassica* plant that is a *B. napus* plant. Though the *B. napus* plant may have a winter flowering habit, one preferred implementation has a spring growing habit, i.e., it does not require vernalization to flower.

#### Production of Hybrid *Brassica* Varieties

Hybrid *Brassica* varieties can be produced by preventing self-pollination of female parent plants (i.e., seed parents), permitting pollen from male parent plants to fertilize such female parent plants, and allowing F<sub>1</sub> hybrid seeds to form on the female plants. Self-pollination of female plants can be prevented by emasculating the flowers at an early stage of flower development. Alternatively, pollen formation can be prevented on the female parent plants using a form of male sterility. For example, male sterility can be cytoplasmic male sterility (CMS), nuclear male sterility, molecular male sterility wherein a transgene inhibits microsporogenesis and/or pollen formation, or be produced by self-incompatibility. Female parent plants containing CMS are particularly useful. CMS can be, for example, of the ogu (Ogura), nap, pol, tour, or mur type. See, for example, Pellan-Delourme and Renard, 1987, *Proc. 7<sup>th</sup> Int. Rapeseed Conf.*, Poznan, Poland, p. 199-203 and Pellan-Delourme and Renard, 1988, *Genome* 30:234-238, for a description of Ogura type CMS. See,

16

Riungu and McVetty, 2003, *Can. J. Plant Sci.*, 83:261-269 for a description of nap, pol, tour, and mur type CMS.

In embodiments in which the female parent plants are CMS, the male parent plants typically contain a fertility restorer gene to ensure that the F<sub>1</sub> hybrids are fertile. For example, when the female parent contains an Ogura type CMS, a male parent is used that contains a fertility restorer gene that can overcome the Ogura type CMS. Non-limiting examples of such fertility restorer genes include the Koseno type fertility restorer gene (U.S. Pat. No. 5,644,066) and Ogura fertility restorer genes (U.S. Pat. Nos. 6,229,072 and 6,392,127). In other embodiments in which the female parents are CMS, male parents can be used that do not contain a fertility restorer. F<sub>1</sub> hybrids produced from such parents are male sterile. Male sterile hybrid seed can be inter-planted with male fertile seed to provide pollen for seed-set on the resulting male sterile plants.

The methods described herein can be used to form single-cross *Brassica* F<sub>1</sub> hybrids. In such embodiments, the parent plants can be grown as substantially homogeneous adjoining populations to facilitate natural cross-pollination from the male parent plants to the female parent plants. The F<sub>1</sub> seed formed on the female parent plants is selectively harvested by conventional means. One also can grow the two parent plants in bulk and harvest a blend of F<sub>1</sub> hybrid seed formed on the female parent and seed formed upon the male parent as the result of self-pollination. Alternatively, three-way crosses can be carried out wherein a single-cross F<sub>1</sub> hybrid is used as a female parent and is crossed with a different male parent that satisfies the fatty acid parameters for the female parent of the first cross. Here, assuming a bulk planting, the overall oleic acid content of the vegetable oil may be reduced over that of a single-cross hybrid; however, the seed yield will be further enhanced in view of the good agronomic performance of both parents when making the second cross. As another alternative, double-cross hybrids can be created wherein the F<sub>1</sub> progeny of two different single-crosses are themselves crossed. Self-incompatibility can be used to particular advantage to prevent self-pollination of female parents when forming a double-cross hybrid.

Hybrids described herein have good agronomic properties and exhibit hybrid vigor, which results in seed yields that exceed that of either parent used in the formation of the F<sub>1</sub> hybrid. For example, yield can be at least 10% (e.g., 10% to 20%, 10% to 15%, 15% to 20%, or 25% to 35%) above that of either one or both parents. In some embodiments, the yield exceeds that of open-pollinated spring canola varieties such as 46A65 (Pioneer) or Q2 (University of Alberta), when grown under similar growing conditions. For example, yield can be at least 10% (e.g., 10% to 15% or 15% to 20%) above that of an open-pollinated variety.

Hybrids described herein typically produce seeds having very low levels of glucosinolates (<30 µmol/gram of de-fatted meal at a moisture content of 8.5%). In particular, hybrids can produce seeds having <20 µmol of glucosinolates/gram of de-fatted meal. As such, hybrids can incorporate mutations that confer low glucosinolate levels. See, for example, U.S. Pat. No. 5,866,762. Glucosinolate levels can be determined in accordance with known techniques, including high performance liquid chromatography (HPLC), as described in ISO 9167-1:1992(E), for quantification of total, intact glucosinolates, and gas-liquid chromatography for quantification of trimethylsilyl (TMS) derivatives of extracted and purified desulfoglucosinolates. Both the HPLC and TMS methods for determining glucosinolate levels analyze de-fatted or oil-free meal.

## Canola Oil

*Brassica* plants disclosed herein are useful for producing canola oils with low ALA content. For example, oil obtained from seeds of *Brassica* plants described herein may have an ALA content of 0.5% to 1.6% (e.g., 0.5 to 1.5%, 0.5 to 1.0%, 0.5 to 0.8%, 0.6 to 1.4%, 0.6 to 1.3%, 0.6 to 1.2%, 0.6 to 1.1%, 0.6 to 1.0%, 0.6 to 0.8%, 0.7 to 1.2%, 0.7 to 1.1%, 0.8 to 1.2%, or 0.8 to 1.0%). In some embodiments, *Brassica* plants described herein produce canola oils with low ALA content (e.g., 0.5 to 1.6%) and low or no total saturated fatty acids. For example, oil obtained from seeds of *Brassica* plants described herein may have an ALA content of 0.5 to 1.5% and a total saturated fatty acid content of 2.5 to 6%, 3 to 5%, 3 to 4.5%, 3.25 to 3.75%, 3.0 to 3.5%, 3.4 to 3.7%, 3.6 to 5%, 4 to 5.5%, 4 to 5%, or 4.25 to 5.25%. The palmitic acid content of such oils can be 2.4 to 3.5% (e.g., 2.5 to 3% or 2.7 to 3.3%). The stearic acid content of such oils can be 0.7 to 2.5% (e.g., 0.8 to 1.7%, 0.9 to 1.5%, or 1.0 to 1.5%).

In some embodiments, an oil has an ALA content of 0.5 to 1.5%, an oleic acid content of 60 to 70% (e.g., 62 to 68%, 63 to 67%, or 65 to 66%), and a total saturated fatty acid content of 5 to 10%. In some embodiments, an oil has an ALA content of 0.6 to 1.5% (e.g., 0.7 to 1.4%, 0.8 to 1.3%, or 0.9 to 1.2%) and an oleic acid content of 71 to 80% (e.g., 72 to 78%, 72 to 76%, 73 to 75%, 74 to 77%, 74 to 78%, or 75 to 80%). The total saturated content of such an oil can be 3 to 8% (e.g., 4 to 6%, 4 to 5.5%, 4 to 5%, 5 to 7%, 6 to 8%, or 7 to 8%). In some embodiments, a canola oil can have an ALA content of 0.5 to 1.5%, an oleic acid content of 85 to 87% (e.g., 86 to 87%), and a total saturated fatty acid content of 5 to 6%. In some embodiments, an oil has an ALA content of 0.5 to 1.5%, an oleic acid content of 81 to 90% (e.g., 82 to 88% or 83 to 87%) oleic acid and a total saturated fatty acid content of 3.5 to 4.5% (e.g., 3.75 to 4.25%, 3.9 to 4.1%, or 4.0%).

Oils described herein can have an eicosenoic acid content of 1.0 to 1.9%. For example, an oil can have an eicosenoic acid content of 1.0 to 1.4%, 1.1 to 1.3%, 1.1 to 1.6%, 1.2 to 1.6%, 1.4 to 1.9%, in addition to a low ALA content.

Oils described herein can have a linoleic acid content of 3.5 to 26%, e.g., 3.7 to 4.5%, 8 to 10%, 9 to 12%, 10 to 13%, 11 to 13%, 12 to 16%, 13 to 16%, 14 to 18%, or 14 to 22%, in addition to a low ALA content.

Oils described herein have an erucic acid content of less than 2% (e.g., less than 1%, 0.5%, 0.2, or 0.1%) in addition to a low ALA content.

The fatty acid composition of seeds can be determined by first crushing and extracting oil from seed samples (e.g., bulk seeds samples of 10 or more seeds). TAGs in the seed are hydrolyzed to produce free fatty acids, which then can be converted to fatty acid methyl esters and analyzed using techniques known to the skilled artisan, e.g., gas-liquid chromatography (GLC) according to AOCS Procedure Ce 1e-91. Near infrared (NIR) analysis can be performed on whole seed according to AOCS Procedure Am-192 (revised 1999).

Seeds harvested from plants described herein can be used to make a crude canola oil or a refined, bleached, and deodorized (RBD) canola oil with a low ALA content. Harvested canola seed can be crushed to extract crude oil and, if desired, refined, bleached and deodorized by techniques known in the art. See, e.g., *Bailey's Industrial Oil and Fat Products*, Volume 5, "Edible Fat and Oil Products: Processing Technologies" (6th Edition, 2005). Briefly, refining refers to removing most if not all free fatty acids and other impurities such as phosphatides or protein substances from a crude oil. One common method of refining involves treating an oil with a strong base, followed by extensive washings with water. Bleaching refers to a process that removes natural pigments

(e.g., carotenoids, chlorophylls, and xanthophylls) and other impurities such as metal cations (e.g., Fe, Cu and Zn). Bleaching can be done by absorbing such pigments and/or cations on a natural bleaching earth or clay, which is usually added to an oil under vacuum and high temperature. Deodorizing refers to the removal of relatively volatile trace components (e.g., ketones, aldehydes, alcohols) from an oil that contribute to flavor, odor, and color. Deodorizing is usually done by injecting steam into an oil heated to high temperatures (e.g., about 470° F. to about 510° F.) under high vacuum (e.g., <5 mm Hg).

In one useful example, the seed can be tempered by spraying the seed with water to raise the moisture to, for example, 8.5%. The tempered seed can be flaked using a smooth roller with, for example, a gap setting of 0.23 to 0.27 mm. Heat may be applied to the flakes to deactivate enzymes, facilitate further cell rupturing, coalesce the oil droplets, or agglomerate protein particles in order to ease the extraction process. Typically, oil is removed from the heated canola flakes by a screw press to press out a major fraction of the oil from the flakes. The resulting press cake contains some residual oil.

Crude oil produced from the pressing operation typically is passed through a settling tank with a slotted wire drainage top to remove the solids expressed out with the oil in the screw 25 pressing operation. The clarified oil can be passed through a plate and frame filter to remove the remaining fine solid particles. Further oil can be extracted from the press cake produced from the screw pressing operation using known solvent extraction techniques, e.g., using commercial n-hexane extraction. The canola oil recovered from the solvent 30 extraction process is combined with the clarified oil from the screw pressing operation, resulting in a blended crude oil.

Free fatty acids and gums typically are removed from the crude oil by heating in a batch refining tank to which food 35 grade phosphoric acid has been added. The acid serves to convert the non-hydratable phosphatides to a hydratable form, and to chelate minor metals that are present in the crude oil. The phosphatides and the metal salts are removed from the oil along with the soapstock. The oil-acid mixture is 40 treated with sodium hydroxide solution to neutralize the free fatty acids and the phosphoric acid in the acid-oil mixture. The neutralized free fatty acids, phosphatides, etc. (soapstock) are drained off from the neutralized oil. A water wash may be done to further reduce the soap content of the oil. The 45 oil may be bleached and deodorized before use, if desired, by techniques known in the art.

Oils obtained from plants described herein can have increased oxidative stability, which can be measured using, for example, an Oxidative Stability Index Instrument (e.g., from Omnia, Inc., Rockland, Mass.) according to AOCS 50 Official Method Cd 12b-92 (revised 1993). Oxidative stability is often expressed in terms of "AOM" hours.

Oils obtained from plants described herein also can have increased flavor stability, which can be measured using, for 55 example, trained test panels in room-odor tests according to Mounts, J. Am. Oil Chem. Soc. 56:659-663, 1979 and the AOCS Recommended Practice Cg 2-83 for the Flavor Evaluation of Vegetable Oils (Methods and Standard Practices of the AOCS, 4th Edition (1989)). The technique encompasses standard sample preparation and presentation, as well as reference standards and method for scoring oils.

## Food Compositions

This document also features food compositions containing the oils described above. For example, oils having a low ALA 60 content (e.g., 0.5 to 1.5%) can be used for food applications or for frying. Oils having a low ALA content in combination with a low (6% or less) or very low (3.5% or less) total

19

saturated fatty acid content can be used to replace or reduce the amount of saturated fatty acids and hydrogenated oils (e.g., partially hydrogenated oils) in various food products such that the levels of saturated fatty acids and trans fatty acids are reduced in the food products. In particular, canola oils having a low ALA content in combination with a low total saturated fatty acid content and a mid or high oleic acid content can be used to replace or reduce the amount of saturated fats and partially hydrogenated oils in processed or packaged food products, including bakery products such as cookies, muffins, doughnuts, pastries (e.g., toaster pastries), pie fillings, pie crusts, pizza crusts, frostings, breads, biscuits, and cakes, breakfast cereals, breakfast bars, puddings, and crackers.

For example, an oil described herein can be used to produce sandwich cookies that contain no or reduced levels of partially hydrogenated oils in the cookie and/or crème filling. In some embodiments, the cookies also have a reduced total saturated fatty acid content. Such cookie compositions can include, for example, in addition to canola oil, flour, sweetener (e.g., sugar, molasses, honey, high fructose corn syrup, artificial sweetener such as sucralose, saccharine, aspartame, or acesulfame potassium, and combinations thereof), eggs, salt, flavorants (e.g., chocolate, vanilla, or lemon), a leavening agent (e.g., sodium bicarbonate or other baking acid such as monocalcium phosphate monohydrate, sodium aluminum sulfate, sodium acid pyrophosphate, sodium aluminum phosphate, dicalcium phosphate, glucano-deltalactone, or potassium hydrogen tartrate, or combinations thereof), and optionally, an emulsifier (e.g., mono- and diglycerides of fatty acids, propylene glycol mono- and di-esters of fatty acids, glycerol-lactose esters of fatty acids, ethoxylated or succinylated mono- and diglycerides, lecithin, diacetyl tartaric acid esters or mono- and diglycerides, sucrose esters of glycerol, and combinations thereof). A crème filling composition can include, in addition to canola oil, sweetener (e.g., powdered sugar, granulated sugar, honey, high fructose corn syrup, artificial sweetener, or combinations thereof), flavorant (e.g., vanilla, chocolate, or lemon), salt, and, optionally, emulsifier.

Canola oils (e.g., with a low ALA, low total saturated fatty acid and low or high oleic acid content) also are useful for frying applications due to the polyunsaturated content, which is low enough to have improved oxidative stability for frying yet high enough to impart the desired fried flavor to the food being fried. For example, canola oils can be used to produce fried foods such as snack chips (e.g., corn or potato chips), French fries, or other quick serve foods.

Oils described herein also can be used to formulate spray coatings for food products (e.g., cereals or snacks such as crackers). In some embodiments, the spray coating can include other vegetable oils such as sunflower, cottonseed, corn, or soybean oils. A spray coating also can include an antioxidant and/or a seasoning.

Oils described herein also can be used in the manufacturing of dressings, mayonnaises, and sauces to provide a reduction in the total saturated fat content of the product. Oils described herein can be used as a base oil for creating structured fat solutions such as microwave popcorn solid fats or canola butter formulations.

#### Plant Breeding

The nucleic acids described herein (e.g., fad3D and fad3E nucleic acids) can be used as markers in plant genetic mapping and plant breeding programs. Such markers may include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA detection (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) or microsatellite, for example. Marker-assisted

20

breeding techniques may be used to identify and follow a desired fatty acid composition (e.g., low linolenic acid) during the breeding process. For example, a nucleic acid described herein, such as the nucleic acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:32, or the complement thereof, can be used to identify one or more individual plants that possess the polymorphic allele correlated with the desired linolenic acid content. Those plants then can be used in a breeding program to combine the polymorphic allele with a plurality of other alleles at other loci that are correlated with a desired variation (e.g., in fatty acid composition). In some embodiments, a fragment of the nucleic acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:32, or the complement thereof, that is at least 50 nucleotides in length can be used to distinguish a modified fad3 allele from a wild-type Fad3 allele (e.g., by allele-specific hybridization or by PCR).

Techniques suitable for use in a plant breeding program are known in the art and include, without limitation, backcrossing, mass selection, pedigree breeding, bulk selection, crossing to another population and recurrent selection. These techniques can be used alone or in combination with one or more other techniques in a breeding program. Thus, each identified plant is selfed or crossed to a different plant to produce seed, which is then germinated to form progeny plants. At least one such progeny plant is then selfed or crossed with a different plant to form a subsequent progeny generation. The breeding program can repeat the steps of selfing or outcrossing for an additional 0 to 5 generations as appropriate in order to achieve the desired uniformity and stability in the resulting plant line, which retains the polymorphic allele. In most breeding programs, analysis for the particular polymorphic allele will be carried out in each generation, although analysis can be carried out in alternate generations if desired.

In some cases, selection for other useful traits is also carried out, e.g., selection for disease resistance. Selection for such other traits can be carried out before, during or after identification of individual plants that possess the desired polymorphic allele.

Marker-assisted breeding techniques may be used in addition to, or as an alternative to, other sorts of identification techniques. An example of marker-assisted breeding is the use of PCR primers that specifically amplify a sequence containing a desired mutation in the fad3D or fad3E sequence.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

50

#### EXAMPLES

In the Tables described herein, the fatty acids are referred to by the length of the carbon chain and number of double bonds within the chain. For example, C14:0 refers to myristic acid; C16:0 refers to palmitic acid; C18:0 refers to stearic acid; C18:1 refers to oleic acid; C18:2 refers to linoleic acid; C18:3 refers to ALA; C20:0 refers to arachidic acid; C20:1 refers to eicosenoic acid; C22:0 refers to behenic acid; C22:1 refers to erucic acid; C24:0 refers to lignoceric acid; and C24:1 refers to nervonic acid. "Total Sats" refers to the total of C14:0, C16:0, C18:0, C20:0, C22:0, and C24:0. Representative fatty acid profiles are provided for each of the specified samples.

Unless otherwise indicated, all percentages refer to wt % based on total wt % of fatty acids (i.e., fatty acid moieties) in the oil as determined by measuring the FAME moieties in accordance with the modified version of ROCS Ce 1c-89 set forth in Example 1.







TABLE 1-continued

Fatty acid profile of harvested M4 generation mutant seed.							
M3B-2558-42	1.247	0.044	0.525	0.000	0.284	0.493	9.111
M3B-2558-43	1.339	0.049	0.521	0.000	0.309	0.128	9.034
M3B-2558-44	1.226	0.049	0.492	0.000	0.277	0.120	8.954
M3B-2558-45	1.259	0.047	0.473	0.015	0.259	0.472	8.638
M3B-2558-46	1.269	0.036	0.551	0.000	0.318	0.110	9.241
M3B-2558-47	1.261	0.038	0.510	0.000	0.291	0.405	9.065
M3B-2558-48	1.282	0.045	0.531	0.000	0.265	0.111	8.980
M3B-2558-49	1.188	0.047	0.493	0.000	0.273	0.114	9.114
M3B-2558-50	1.215	0.042	0.503	0.000	0.272	0.431	9.462

Selected M4 individuals were self pollinated to generate M5 seeds and further evaluated in an environmentally controlled plant growth chamber. Seeds from M3B-2558-35 and M3B-1904-35 were planted in Premier Pro-Mix BX potting soil (Premier Horticulture, Quebec, Canada) in four inch plastic pots. Planted seeds were watered and stratified at 5°C. for 5 days and germinated at 20°C. day temperature and 17°C. night temperature (20/17) in Conviron ATC60 controlled-environment growth chambers (Controlled Environments Limited, Winnipeg, MB). Each genotype combination was randomized and replicated 10 times in each of two separate growth chambers. At flowering, one chamber was reduced to a diurnal temperature cycle of 15°C. day temperature and 12°C. night temperature (15/12) while the other remained at 20/17. The temperature treatments were imposed to identify the effects of temperature on fatty acid composition. Plants were watered five times per week and fertilized bi-weekly using a 20:20:20 (NPK) liquid fertilizer at a rate of 150 ppm. Plants were bagged individually to ensure self pollination and genetic purity of the seed. Seeds from each plant were harvested individually at physiological seed maturity. The fatty acid profile of the seeds was determined using the modified GC method described above (replicates of two).

Fatty acid data from plants grown under the different temperature regimes was analyzed in two ways. First, data was analyzed separately as different environments and then it was pooled and analyzed across environments. Data was analyzed in SAS (SAS Institute, 2003) using proc glm to estimate differences in mean fatty acid values. Table 2 contains the population size, mean value and standard deviation of oleic, linoleic and linolenic fatty acid of seeds produced by plants carrying mutant fad3 alleles and grown in two environmental growth chambers set at different diurnal temperature regimes (20°C. day/17°C. night; 15°C. day/12°C. night) as discussed above. Genotypes 1904-35 and 2558-35 are mutant allele combinations and v1030 hybrid and IMCO2 are controls. The 1904-35, 2558-35, and IMCO2 lines each contain mutant fad3A and fad3B alleles, while line 1904-35 also contains a mutant fad3E allele and a mutant fad3D allele (see below). Means with different letters are significantly different as determined by a Student-Newman-Keuls mean separation test. In conclusion, lines 1904-35 and 2558-35 can reach an alpha-linolenic content less than v1030 and IMCO2.

Seeds of lines 1904 and 2558 were deposited with the American Type Culture Collection (ATCC) (Manassas, Va.) on Sep. 1, 2010, under conditions of the Budapest Treaty and assigned Accession Nos. PTA-11273 and PTA-11274, respectively. All restrictions upon public access to the deposits will be irrevocably removed upon grant of the patent. The deposits will be replaced if the depository cannot dispense viable samples.

TABLE 2

Mean oleic, linoleic and linolenic acid content in two environments							
RESCHID	Mean C18:1	s.d.	Mean C18:2	s.d.	Mean C18:3	s.d.	N
15/12 Environment							
v1030	65.877	0.564	22.031	0.523	3.430 a	0.116	9
IMCO2	69.728	1.528	20.484	1.434	1.815 b	0.109	9
1904-35	73.986	1.437	16.956	1.369	1.071 c	0.082	10
2558-35	77.276	1.191	13.051	1.505	0.976 d	0.081	10
17/20 Environment							
v1030	65.053	1.397	22.906	1.570	2.952 a	0.133	10
IMCO2	72.211	1.604	17.543	1.986	1.378 b	0.098	10
1904-35	77.009	0.475	13.477	0.489	1.052 c	0.040	9
2558-35	78.470	0.924	11.238	1.129	0.993 c	0.080	10
Across Environments							
V1030	65.443	1.138	22.491	1.247	3.179 a	0.274	19
IMCO2	71.035	1.987	18.936	2.272	1.585 b	0.246	19
1904-35	75.418	1.881	15.308	2.056	1.062 c	0.065	19
2558-35	77.873	1.205	12.145	1.595	0.984 c	0.079	20

## Example 2

## Identification of a Fad3E Mutation in 1904-35 Plants

Genome mapping, map-based gene cloning, and direct-sequencing strategies were used to identify loci associated with the <1.5% linolenic fatty acid content in the 1904-35 line described in Example 1. A DH (doubled haploid) population was developed from a cross between 1904-35 and 95CB504, a B line (maintainer). The two parental lines were screened with 1066 SNP (single nucleotide polymorphism) markers using the MassARRAY platform (Sequenom Inc., San Diego, Calif.) to identify polymorphic SNP markers between the two parents; 174 polymorphic SNP markers were identified.

Single marker correlations and multiple regression analysis between fatty acid composition and SNP markers were carried out using the SAS program (SAS Institute 1988). A *Brassica napus* genetic linkage map was constructed using the Kosambi function in JoinMap 3.0 (Kyazma). Interval mapping for quantitative trait loci (QTL) was done with MapQTL 4.0 (Kyazma). A LOD score >3.0 was considered as the significance threshold to declare the association intervals.

Comparative genome mapping was performed to locate the identified QTL in *Brassica napus* chromosomes and further identify the *Brassica rapa* BAC (Bacterial Artificial Chromosome) clones encompassing the identified SNP markers and the candidate genes in the identified QTL interval for the <1.5% linolenic acid content using publicly available *Brassica* and *Arabidopsis* genome sequences, genes, genetic linkage maps, and other information from the world wide web at brassica.bbsrc.ac.uk/, and ncbi.nlm.nih.gov/.

A total of 217 DH lines were genotyped with 174 polymorphic SNP markers. QTL mapping identified two QTLs for low linolenic acid content (<1.5% C18:3). Comparative genome mapping located one QTL on the N3 chromosome in *Brassica napus* (A3 in *Brassica rapa*) and further identified a Fad3E candidate gene which is located at 1cM from the SNP marker that showed significant association with C18:3 content. The 1cM interval between the SNP marker and Fad3E gene is 248 kb according to co-linearity with the *Arabidopsis* genome. Example 3 describes the second QTL on the N5 chromosome in *Brassica napus* (A5 in *Brassica rapa*).

The Fad3E genes from chromosome N3 of the *Brassica napus* genome were sequenced from 1904-35, 95CB504 and IMC201. The sequences were analyzed using BLAST (the Basic Local Alignment Search Tool) and DNASTAR/Lasergene 8.0 (DNASTAR, Inc.). A single nucleotide substitution was identified in one of the two Fad3E isoforms from the 1904-35 mutant line that was not present in 95CB504 and IMC201. FIG. 1 shows the sequence alignment of the BnFad3E gene from 1904-35 and IMC201, and the BrFad3E located in *Brassica rapa* BAC, KBrH013B15 from the world wide web at brassica-rapa.org. The nucleotide substitution of a "A" in 1904-35 for "G" in IMC201 and 95CB504 at position 1851 of this alignment (position 1756 in SEQ ID:NO:1). As shown in FIG. 2, this transition mutation of Fad3E is at the exon 3, intron 3 border. FIG. 3 shows the alignment of FAD3E amino acid sequences from 1904 BnFAD3E-2 and IMC201 BnFAD3E-2 (SEQ ID NO:29), BrFAD3E deduced from BrFad3E (world wide web at brassica-rapa.org) (SEQ ID NO:30), and AtFAD3 (GenBank accession number: NP\_180559; SEQ ID NO:31). The fad3E-2 SNP allele results in an altered consensus sequence at the "splice donor site" for RNA splicing. Therefore, the RNA splicing of fad3E-2 primary transcript (pre-mRNA) cannot be processed to create a mature RNA (mRNA).

Large scale screening of the parental lines (1904-35 and 95CB504) as well as other *Brassica napus* cultivars including 2558, indicated the fad3E-2 SNP allele was 1904-35-specific and was significantly associated with the low ALA phenotype (R-square=0.275 for C18:3 content) using 217 DH lines developed from the cross between 1904-35 and 95CB504. This 1904-35 fad3E-2 SNP allele also was present in selections having <1.5% C18:3 content from a backcross population developed from the cross between 1904-35 and 1035R, an R line (restorer).

### Example 3

#### Identification of a Fad3D Mutation in 1904-35 Plants

As indicated in Example 2, a 2<sup>nd</sup> QTL was also identified for low linolenic acid content. Comparative genomics located this 2nd QTL on the N5 chromosome of *Brassica napus* and further identified a Fad3D candidate gene on chromosome N5. The Fad3D genes from chromosome N5 of the *Brassica napus* genome were sequenced from 1904-35, 95CB504, and IMC201. The sequences were analyzed using BLAST and DNASTAR/Lasergene 8.0 (DNASTAR, Inc.). FIG. 4 shows the sequence alignment of a portion of the BnFad3D gene from 1904-35, 95CB504 and IMC201.

A deletion was identified in one of the two Fad3D isoforms from the 1904-35 mutant line that was not present in 95CB504 and IMC201. The mutant type BnFad3D from 1904-35 has a deletion including a portion of exon 1 (from position 575 to position 739). In IMC201 and 95CB504, exon 1 starts at position 441 and ends at position 739. As a result of the deletion in 1904-35, exon 1 is only 134 bp long. There-

fore, it is believed the deletion mutation in 1904 BnFad3D induced a non-functional truncated protein/enzyme due to either lack of RNA splicing (truncated protein with 64 amino acids) or incorrect RNA splicing (truncated protein).

Large scale screening of the parental lines (1904-35 and 95CB504) as well as other *Brassica napus* cultivars, indicated the Fad3D deletion was 1904-35-specific. In addition, the Fad3D deletion was significantly associated with the low ALA phenotype (R-square=0.61, equal to 61% phenotypic variation on C18:3) using the parental lines and 77 DH lines developed from the cross between 1904-35 and 95CB504 compared with 22% explained by BnFad3E-2 mutation in 1904-35. In order to determine the relative effect of individual Fad3 isoform on C18:3 content, 215 lines were used from multiple populations, which carry all Fad3 isoforms, for the multiple regression analysis. Results demonstrated that BnFad3B explains the largest proportion of phenotypic variation on C18:3 content with 26%, followed by 16% by BnFad3D, 8% by BnFad3A, and 7% by BnFad3E.

### Example 4

#### Mutant Fad3A and Fad3B Genes

A population of *B. napus* IMC201 seeds was subjected to chemical mutagenesis as set forth in Example 1. Approximately 200,000 treated seeds were planted in standard greenhouse potting soil and placed into environmentally controlled greenhouse. The plants were grown under sixteen hours of day light. At maturity, M2 seed was harvested from the plants and bulked together. The M2 generation was planted and leaf samples from the early, post-cotyledon stage of development from 8 plants were pooled and DNA was extracted from leaves of these plants. The leaf harvest, pooling and DNA extraction was repeated for approximately 32,000 plants, and resulted in approximately forty 96-well blocks containing mutagenized *B. napus* IMC201 DNA. This grouping of mutagenized DNA is referred to below as the original DNA mutagenesis library.

Additionally, approximately 200,000 treated seeds from the dual mutagen treatment described in Example 1 were planted in standard greenhouse potting soil and placed into environmentally controlled greenhouse. The plants were grown under sixteen hours of day light. At maturity, M2 seed was harvested from the plants and bulked together. This M2 generation was planted in greenhouses and, at flowering, plants were bagged in groups of four to facilitate cross-pollination that would occur in parallel with the majority self pollination events, and seed from this generation was harvested. Genomic DNA from three seeds per plant of this M3 generation was isolated in 96-well blocks; a collection of mutagenized DNA from this process is referred to below as the new Tilling DNA mutagenesis library.

The original DNA mutagenesis library and the new Tilling DNA mutagenesis library were screened to identify stop-codon containing fad3A and fad3B mutant alleles. PCR reactions were performed using *B. napus* IMC201 genomic DNA original mutagenesis library or new Tilling DNA mutagenesis library. PCR products from the original mutagenesis library were analyzed using temperature gradient capillary electrophoresis on a REVEAL® instrument (Transgenomics Inc.), which allows PCR reactions containing heterogeneous PCR products to be distinguished from reactions containing only homogeneous products, as would be the case if a SNP existed in genomic DNA from chemical mutagenesis and subsequent PCR amplification. The PCR products from the new Tilling DNA mutagenesis library were sequenced directly using an

**31**

Applied Biosystems (Life Technologies) 3730 DNA sequencer using the manufacturer's recommendations.

Individual seeds representing the primary hit of each M2 plant that was the source genomic DNA mix for this primary mutagenesis screen were sampled and genomic DNA was isolated in order to perform the Fad3A PCR on these individuals. PCR products were sequenced and the sequences were compared to the wild-type sequence to screen for the presence of an induced stop codon.

The sequence comparisons indicated that a mutation had been generated and mutant plants obtained for each of the Fad3A and Fad3B genes. The mutant Fad3A sequence is shown in SEQ ID NO: 9 and contains a mutation at position 102, changing the codon from TGG to TGA. The mutant Fad3B sequence is shown in SEQ ID NO:10 and contains a mutation at position 206, resulting in a codon change from TGG to TAG.

**32**

Example 5

DH Line Husker

A cross was made between 1904-35 (Example 1) and 95CB504, a B line (maintainer). A double haploid population was generated by collecting  $F_1$  microspores from the cross, treating the microspores with colchicine, and propagating them *in vitro*. Plantlets formed *in vitro* from the microspores were moved to a greenhouse and inflorescences that formed were self pollinated. Seed was harvested from the DH<sub>1</sub> plants at maturity and analyzed for fatty acid profile. Seeds from those plants exhibiting low and high linolenic acid content were grown in the greenhouse. Table 3 contains the fatty acid profile of a bulk sample of seeds produced by each of 5-10 greenhouse-grown plants of a DH<sub>1</sub> population designated Husker.

TABLE 3

Fatty acid profile of DH <sub>1</sub> population designated Husker										
RESCHID	Pedigree	n	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0
Husker-100	95CB504xM3B-1904-35	5	0.00	2.308	0.078	1.728	80.758	11.130	0.766	0.774
Husker-141	95CB504xM3B-1904-35	3	0.00	3.353	0.140	2.310	78.293	11.920	0.923	0.887
Husker-147	95CB504xM3B-1904-35	5	0.03	3.336	0.160	2.048	78.186	11.984	0.858	0.938
Husker-161	95CB504xM3B-1904-35	5	0.03	3.688	0.242	2.246	78.816	10.864	0.814	0.920
Husker-107	95CB504xM3B-1904-35	5	0.04	4.130	0.256	2.070	75.004	12.408	2.444	0.934
Husker-125	95CB504xM3B-1904-35	5	0.02	3.363	0.175	2.080	78.211	11.661	1.161	0.891
Husker-138	95CB504xM3B-1904-35	5	0.02	3.574	0.195	2.151	77.702	11.767	1.240	0.914
Husker-170	95CB504xM3B-1904-35	4	0.04	4.768	0.268	1.808	75.962	11.738	2.336	0.780
Husker-314	95CB504xM3B-1904-35	5	0.01	2.765	0.153	1.900	79.270	12.533	0.738	0.718
Husker-323	95CB504xM3B-1904-35	5	0.03	4.526	0.168	2.370	75.004	11.948	2.338	0.934
95CB504		9	0.05	3.723	0.231	2.518	78.586	9.073	2.256	1.030
RESCHID	Pedigree	n	C20:1	C20:2	C22:0	C22:1	C24:0	C24:1	TOT SATS	
Husker-100	95CB504xM3B-1904-35	5	1.468	0.030	0.442	0.008	0.266	0.248	5.516	
Husker-141	95CB504xM3B-1904-35	3	1.227	0.000	0.447	0.000	0.233	0.273	7.227	
Husker-147	95CB504xM3B-1904-35	5	1.346	0.040	0.550	0.000	0.310	0.224	7.210	
Husker-161	95CB504xM3B-1904-35	5	1.330	0.016	0.484	0.004	0.304	0.244	7.670	
Husker-107	95CB504xM3B-1904-35	5	1.474	0.030	0.598	0.006	0.364	0.242	8.140	
Husker-125	95CB504xM3B-1904-35	5	1.369	0.023	0.504	0.004	0.295	0.246	7.153	
Husker-138	95CB504xM3B-1904-35	5	1.349	0.022	0.517	0.003	0.301	0.246	7.480	
Husker-170	95CB504xM3B-1904-35	4	1.344	0.018	0.478	0.000	0.232	0.224	8.108	
Husker-314	95CB504xM3B-1904-35	5	1.150	0.038	0.338	0.000	0.170	0.220	5.903	
Husker-323	95CB504xM3B-1904-35	5	1.568	0.034	0.538	0.010	0.324	0.208	8.722	
95CB504		9	1.446	0.040	0.533	0.017	0.321	0.189	8.163	

# US 9,185,861 B2

**33**  
Example 6

## DH Line Vest

A cross was made between 2558-35 (Example 1) and Dumpling-314, a double haploid B-line (maintainer) developed from a cross between IMC106RR and Jetton, a known winter rapeseed variety. A double haploid population was generated as described in Example 5. Seed was harvested from the DH<sub>1</sub> plants at maturity and analyzed for fatty acid profile. Seeds from those plants exhibiting low linolenic acid content were grown in the greenhouse. Table 4 contains the fatty acid profile of a bulk sample of seeds produced by each of 10 greenhouse-grown plants of a DH<sub>1</sub> population designated Vest.

**34**  
Example 7

## Development of Hybrid Canola Producing Reduced ALA in the Seed Oil

A hybrid canola line yielding seeds with an ALA content of less than 1.5% was produced by introducing genes from line 1904-35 (Example 1) into a commercially grown hybrid variety, Victory® v1035. Hybrid v1035 has an average oleic acid content of 65% and an ALA content of 2.8%. Plants of the line 1904-35, and the inbreds 1035R and 95CB504, were planted in a greenhouse. Inbred 1035R is the male parent of v1035. Inbred 95CB504 is the B line female parent of v1035. Plants of 1035R and 1904-35 were cross pollinated in the greenhouse, as were 95CB504 and 1904-35, as shown in Table 5.

TABLE 4

Mean fatty acid profile of Vest DH lines from tails of C18:3 distribution (Vest Population N = 51)

Research ID	Pedigree	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0
Vest-70	Dump314-05xM3B-2558-35-2	0.069	4.507	0.223	1.983	67.152	22.456	0.653	0.778
Vest-52	Dump314-05xM3B-2558-35-2	0.060	4.001	0.308	1.844	77.279	12.080	0.680	0.876
Vest-86	Dump314-05xM3B-2558-35-2	0.065	4.406	0.264	2.266	79.352	9.008	0.745	1.009
Vest-60	Dump314-05xM3B-2558-35-2	0.072	4.685	0.273	3.143	77.323	10.143	0.787	1.217
Vest-87	Dump314-05xM3B-2558-35-2	0.082	5.083	0.346	2.317	68.253	18.677	0.790	1.001
Vest-75	Dump314-05xM3B-2558-35-2	0.101	5.060	0.442	2.760	70.842	14.678	1.830	1.142
Vest-69	Dump314-05xM3B-2558-35-2	0.143	5.840	0.653	3.079	65.526	18.353	1.952	1.359
Vest-71	Dump314-05xM3B-2558-35-2	0.103	5.719	0.515	4.595	57.872	23.001	2.039	1.673
Vest-92	Dump314-05xM3B-2558-35-2	0.137	6.678	0.606	3.026	54.170	28.786	2.423	1.300
Vest-97	Dump314-05xM3B-2558-35-2	0.126	6.439	0.643	3.483	54.626	27.270	2.528	1.315
Dumpling-314 avg (n = 4)		0.051	4.405	0.255	2.259	67.459	20.564	1.396	0.969
Research ID	Pedigree	C20:1	C20:2	C22:0	C22:1	C24:0	C24:1	TOT SATS	
Vest-70	Dump314-05xM3B-2558-35-2	1.191	0.066	0.431	0.048	0.249	0.193	8.017	
Vest-52	Dump314-05xM3B-2558-35-2	1.562	0.053	0.571	0.043	0.411	0.233	7.762	
Vest-86	Dump314-05xM3B-2558-35-2	1.486	0.044	0.623	0.044	0.468	0.220	8.838	
Vest-60	Dump314-05xM3B-2558-35-2	1.287	0.042	0.597	0.000	0.430	0.000	10.145	
Vest-87	Dump314-05xM3B-2558-35-2	1.284	0.064	0.624	0.000	0.505	0.976	9.612	
Vest-75	Dump314-05xM3B-2558-35-2	1.415	0.067	0.657	0.000	0.516	0.491	10.235	
Vest-69	Dump314-05xM3B-2558-35-2	1.324	0.111	0.842	0.000	0.626	0.192	11.889	
Vest-71	Dump314-05xM3B-2558-35-2	1.125	0.085	1.005	0.000	0.660	1.608	13.755	
Vest-92	Dump314-05xM3B-2558-35-2	1.191	0.000	0.695	0.000	0.628	0.359	12.464	
Vest-97	Dump314-05xM3B-2558-35-2	1.088	0.000	0.712	0.441	0.616	0.715	12.690	
Dumpling-314 avg (n = 4)		1.380	0.062	0.571	0.016	0.454	0.160	8.708	



TABLE 8-continued

GP #1-24	Vest-57x(1764-43-6x1975-90-14)	0.05	3.11	0.29	0.95	74.63	16.92	1.06	0.47
GP #1-181	Vest-57x(1764-43-6x1975-90-14)	0.04	3.11	0.26	1.02	75.24	16.41	1.06	0.46
GP #1-444	Vest-57x(1764-43-6x1975-90-14)	0.03	2.78	0.23	1.15	76.61	14.89	0.99	0.53
GP #1-449	Vest-57x(1764-43-6x1975-90-14)	0.03	3.02	0.25	1.11	77.22	14.22	1.08	0.51
GP #1-240	Vest-57x(1764-43-6x1975-90-14)	0.00	2.80	0.20	1.35	74.47	16.93	1.04	0.55
GP #1-150	Vest-57x(1764-43-6x1975-90-14)	0.04	2.59	0.19	1.54	79.41	12.02	0.97	0.63
GP #4-20	Vest-70x(1764-43-6x1975-90-14)	0.02	2.74	0.17	1.28	75.26	16.19	1.04	0.55
GP #4-17	Vest-70x(1764-43-6x1975-90-14)	0.03	3.48	0.16	1.22	65.47	25.83	0.82	0.51
GP #5-434	(01OB054RxLSAt15.36)xVest-57-05	0.03	2.83	0.13	1.56	77.51	13.35	1.09	0.66
GP #5-*	(01OB054RxLSAt15.36)xVest-57-05	0.03	2.99	0.23	1.31	86.21	4.37	1.11	0.67
GP #5-351	(01OB054RxLSAt15.36)xVest-57-05	0.02	3.47	0.00	1.43	76.99	14.12	0.86	0.60
GP #5-334	(01OB054RxLSAt15.36)xVest-57-05	0.03	3.16	0.18	1.52	73.74	16.87	1.05	0.62
GP #5-404	(01OB054RxLSAt15.36)xVest-57-05	0.03	2.96	0.15	1.61	79.04	11.59	1.08	0.70
GP #5-332	(01OB054RxLSAt15.36)xVest-57-05	0.04	3.16	0.22	1.61	86.99	3.90	1.02	0.66
GP #5-344	(01OB054RxLSAt15.36)xVest-57-05	0.04	2.92	0.23	1.71	86.45	4.10	0.97	0.79
	(1764-43-6x1975-90-14) avg (n = 12)	0.03	2.89	0.21	1.17	70.30	18.55	2.84	0.61
	Vest-57 avg (n = 10)	0.04	4.17	0.23	1.67	77.79	11.35	0.91	0.83
	Vest-70 avg (n = 12)	0.06	4.76	0.21	1.60	65.52	23.50	0.95	0.74
Research ID	Pedigree	C20:1	C20:2	C22:0	C22:1	C24:0	C24:1	TOT SATS	
GP #1-396	Vest-57x(1764-43-6x1975-90-14)	1.67	0.10	0.34	0.05	0.21	0.20	4.72	
GP #1-24	Vest-57x(1764-43-6x1975-90-14)	1.60	0.07	0.33	0.04	0.17	0.30	5.08	
GP #1-181	Vest-57x(1764-43-6x1975-90-14)	1.55	0.07	0.32	0.04	0.19	0.22	5.15	
GP #1-444	Vest-57x(1764-43-6x1975-90-14)	1.72	0.07	0.40	0.04	0.27	0.27	5.16	
GP #1-449	Vest-57x(1764-43-6x1975-90-14)	1.68	0.08	0.34	0.03	0.18	0.25	5.20	
GP #1-240	Vest-57x(1764-43-6x1975-90-14)	1.64	0.10	0.38	0.06	0.25	0.24	5.32	
GP #1-150	Vest-57x(1764-43-6x1975-90-14)	1.67	0.09	0.38	0.03	0.22	0.23	5.39	
GP #4-20	Vest-70x(1764-43-6x1975-90-14)	1.76	0.10	0.35	0.06	0.26	0.23	5.21	
GP #4-17	Vest-70x(1764-43-6x1975-90-14)	1.58	0.11	0.34	0.05	0.16	0.25	5.74	
GP #5-434	(01OB054RxLSAt15.36)xVest-57-05	1.76	0.10	0.44	0.05	0.24	0.25	5.76	
GP #5-*	(01OB054RxLSAt15.36)xVest-57-05	1.92	0.05	0.52	0.05	0.29	0.26	5.80	
GP #5-351	(01OB054RxLSAt15.36)xVest-57-05	1.71	0.06	0.34	0.04	0.19	0.18	6.04	
GP #5-334	(01OB054RxLSAt15.36)xVest-57-05	1.69	0.10	0.41	0.05	0.31	0.26	6.05	
GP #5-404	(01OB054RxLSAt15.36)xVest-57-05	1.74	0.08	0.49	0.05	0.29	0.18	6.08	
GP #5-332	(01OB054RxLSAt15.36)xVest-57-05	1.44	0.05	0.44	0.05	0.27	0.15	6.18	
GP #5-344	(01OB054RxLSAt15.36)xVest-57-05	1.76	0.07	0.48	0.04	0.24	0.20	6.19	
	(1764-43-6x1975-90-14) avg (n = 12)	1.92	0.15	0.50	0.12	0.35	0.37	5.54	
	Vest-57 avg (n = 10)	1.57	0.06	0.63	0.06	0.42	0.28	7.75	
	Vest-70 avg (n = 12)	1.40	0.09	0.52	0.06	0.33	0.28	8.01	

**39**

## OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description

**40**

thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

## SEQUENCE LISTING

&lt;160&gt; NUMBER OF SEQ ID NOS: 34

&lt;210&gt; SEQ ID NO 1

&lt;211&gt; LENGTH: 3691

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Brassica napus

&lt;400&gt; SEQUENCE: 1

atgggttgttgc	ctatggacca	acgcaccaat	gtgaacggag	atgcccgtgc	ccgaaaggaa	60
gaagggttttg	atccgagcgc	acaaccgcgc	ttaagatcg	gggacataag	ggctgcgatt	120
cctaagcatt	gttgggtgaa	aagtcccttg	agatctatga	gtcacgtgc	cagagacatt	180
tgtgcgcgtcg	cggctttggc	cattgccgc	gtgtattttgc	atagctggtt	cctctgtct	240
ctctattggg	tcgccccagg	aacccttttc	tggccatct	tcgtccctcg	ccacgactgg	300
taaagtttct	tccattttgc	attgcatoga	tttattgaat	gcacgttcta	cgagtattgt	360
ttgtcagttt	cttcgtaaaa	tgttcttttgc	gatgttcat	ttttgaagat	ctaatgtttt	420
tttttttaga	tttttttttttt	aaatcattgt	tccaccacca	ccttcatcg	gtcgta	480
tcgttacaac	accacatctt	tatttctat	aattactact	gttccgcac	tttatggatc	540
tctcaactta	taattaaagt	ataatatcaa	gaatatctat	tattttctt	aaacaagaaa	600
gataatatttgc	ttttttttttttt	attttgggtgt	atttccaatc	tatccgaga	tttagaaatgt	660
tgacacgtca	ttaccttgc	gaagtgttta	aaacaaacat	ggaaagtttgc	aataaataatgt	720
gcaataaatgc	atatatatgt	atatgtatgc	taatgtatgt	aaatataatt	gaataatggc	780
agtggacatg	ggagtttctc	agacattcct	ctgctgaata	gtgtgggtgg	ccatattttt	840
cattccttca	tcctcggtcc	ttaccatgttgc	tggtaagtca	gtttatcaac	cctttttact	900
atattattaa	ttattaaact	tgcatttgta	tacttggtgc	aagttggtaa	atgtaatctgc	960
ataactgaaa	atctattcat	tgctcggttct	atttttttttttt	tggctagaga	caattttata	1020
attaataat	gcatgtgaga	atatgactat	ttatgtgagg	tagttttct	tattctgtc	1080
gaaaagcatc	aatcttttag	caacgaagga	aaaaggaatc	aaattttttgc	ttaaatgcaaa	1140
tgggtctatg	tcttggtcat	tagtttttgc	catataat	atttatattt	ttttcttac	1200
agcagctaat	ttaattataa	ttaaatatcc	atttataaaa	taatatttgc	ccaatttata	1260
agggttagat	attttaagaa	ttattcatga	ctttgtttat	tggactcct	tttatctttt	1320
aatctttctt	atttcttccat	ttttataat	gagaaactgac	cttcaaatct	ccaataaaaga	1380
tggtcttgc	tagtaacagt	ataatttttttgc	gtttggtaaa	tgtacatca	tcttcaatata	1440
tctttgaaaa	tagacttaca	tgcattttttgc	tgctgcgaca	ttattgtcac	ttattcttgc	1500
caataaaat	gtttattact	gaacttttttgc	ttggtaattt	tattactgt	aactttaaac	1560
ttaaaagagt	gagattgttgc	gtcaaaaaata	gagtggata	gttagaatct	1620	
gccatgaaag	caacactata	tagacaat	aatttttgc	aaaacacatt	taataat	1680
aggctgcagg	agaataagcc	atcgacaca	ccaccagaac	catggccatg	ttgaaaacg	1740
cgagtcttgg	gttccagtaa	cattccctc	ttaataatttgc	tctatcttgc	tgtcaaaata	1800
attagtttttgc	cgaaatttgc	ggccagaacgc	accacttgc	aaatttgatttgc	tttagctgt	1860
gtaaaaacag	tttgcgtatgc	tcacagttaa	ccggtaatttgc	attcttttgc	acgatatttgc	1920

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gaagtaacat ttttgtaaaa taaaatatac attatggat gtgacaacgg accacgctt	1980
tttgtattgg tgaatctttt aattactccc tccaatttat tttagtgca gattttagatt	2040
tatgcacata gattaataaa aatattttgc acatttcaa aataaaaaca ccattactt	2100
tacaactaac cataattcaa ccaataaaaa taaaattagaa aatattattt ataaaatttg	2160
tattgaaatt ataaaataat acttatttta aaacgaaatt aatttacaac gacaattaaa	2220
ctgaaacgga aagaattat taatacttaa ttaaagagg ttttagaaaa ttgaaagaca	2280
tgtttatgcg aaactcatgt gaaagtctt gaaataatag atttggat aaatattca	2340
aattttctta aaataataat tatataattaa tataatttg gataaaatct cgtcaaaaac	2400
tcactaatgc aaatgctttt atttgaatt tcttactcct ctaaatgcat ttactttat	2460
actaatatta ttttcttct ctaatttgc gttcgtat agttgtctg tattttgaaa	2520
actaacaaaa aataataaaa acaaagctt ataaacacat agcatgcaat gaatatgtac	2580
gaatatataat accaatacat atctaagtac tattttccca agtacttaat ctgttattact	2640
aaaattcatt ttaattgttc ct当地cgtt ccagaaagg tatacaagaa tttaccac	2700
agtactcgga tgctcagata cactgtccct ct当地catgc tc当地taccc gatctatctg	2760
gtattttta attcctaaaa ttactacaa gtcattttag actgtgtttt aaaacaat	2820
aattttttt gtttggttt actgcagtgg tacagaagtc ctggaaaaga agggtcacat	2880
tttaaccat acagtggttt atttgctcca agcgagagaa agcttattgc aacttcgact	2940
acttgctggt ccataatgtt ggcaattttt atctgtctt cttccctcg tggccagtc	3000
acagttctca aagtatacgg tggcccttac attgttaattt tcttagtata tcataaagg	3060
tatataattt ttattcaata tataactat atgattttt tttgtcatat attttgaaa	3120
tattcagatc tttgtatgtt gggtggacgc tgcacttac ttgcattacc atggcatga	3180
tgagaagttt ctttgcata gaggcaagg aattaaatca actattacaa gtattttaca	3240
aaaaactaat gattgtata ttgttattt ctttattttt gatgtttttt gattaataat	3300
aggaatggag ttacttacgt ggaggattaa caactattga tagagattac ggaattttca	3360
acaacattca tcacgacatt ggaactcagc tgatccatca tttttccca caaatccctc	3420
actatcattt ggtcgatgtt gtgagtcattt tcactctctg gttttttca tcaaaaccat	3480
ttgattaaag ggtgatttt tactaatgtt gtgattttaa caaatggaaat gtgacagaca	3540
aaagcagctt aacatgtgtt gggagataac tacagagaac caaagacgtc aggagcaata	3600
ccgatccact tggggagag tttggtagca agtattaaga aagatcatta cgtcagtgc	3660
actggtgaca ttgttttca cgagactgat c	3691

<210> SEQ\_ID NO 2  
<211> LENGTH: 3691  
<212> TYPE: DNA  
<213> ORGANISM: Brassica napus

&lt;400&gt; SEQUENCE: 2

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gaagggtttt atccgagcgc acaaccggcc tttaagatcg gggacataag ggctgcgatt	120
cctaaggattt gttgggtgaa aagtcctttt agatctatgtt gctacgtac cagagacatt	180
tgtgccgtcg cggctttggc cattggccgc gtgtattttt atagctggtt cctctgtcct	240
ctctattggg tcgccccagg aacccttttcc tggggccatct tcgtcctcgg ccacgactgg	300

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taaagtttct tccatTTgc attgcatacg aTTattGAAT gcacGTTcta CGAGTATTGT	360
ttgtcagTTA CTTCGTAaaa tgattCTTT gatgttcatt ttttGAAGAT ctaAGATTT	420
ttttttAGA TTTCTTTT aaATCATTGT TCCACCAcCA CCTTCATCG GTCGTACGAC	480
tcgttacaAC accACATCTT tATTTCTAT aATTACTACT GCTTCCGcat TTTATGGATC	540
tCTCAACTTA TAATTAAGT ATAATATCAA GAATATCTAT TATTTTCTT AAACAAGAAA	600
GATAATATTG TTTCTTGTT ATTTGGTGT ATTTCCAATC TATTTCGAGA TTTAGAAATG	660
TGACACGTCA TTACCTTGTt GAAGTGTtTA AAACAAACAT GGAAAGTTA AATAAATAGT	720
gcaATAAATG ATATATATGT ATATGATGAA TAATGATGTG AAATATAATT GAATAATGGC	780
AGTGGACATG GGAGTTCTC AGACATTCT CtgctgaATA GTGTGGTTG CCATATTCTT	840
CATTCCtCA TCCTCGTCC TTACCATGGT TGGTAAGTCA GCTTATCAAC CCTTTTACT	900
ATATTATTAa TTATTAACt TGCAATTGTA TACTTGGTGC AAGTGGTAA ATGTAATCTG	960
ATAACTGAAA ATCTATTCTAT TGCTCGTTCT ATTtTTTTT TGGCTAGAGA CAATTTATA	1020
ATTAATAAAT GCATGTGAGA ATATGACTAT TTATGTGAGG TAGCTTTCT TATCCTGTc	1080
GAAAAGCATC AAATCTTtAG CAACGAAGGA AAAAGGAATC AAATTTTTA TTAAATGCAA	1140
TGGGTCTATG TCTTGGTCAt TAGTTTTG CATATAATT ATTtATATTt TTtTTTAAC	1200
AGCAGCTAAT TTAATTATAA TTAAATATTc ATTtATAAA TAATATTAGA CCAATTATTa	1260
AAGGTTAGAT ATTtTAAGAA TTATTCTATGA CTTTGTtTA tGGAACTCCT TTTATCTTT	1320
AAATCTTCT ATTtCTCCAT TTtTAATAAT GAGAAACTGA CTTCAAATCT CCAATAAAGA	1380
TGGTCTTATG TAGTAACAGT ATAATTTTT GTTTGGTAAAG TGTAAACATCA TCTTCAAATA	1440
TCTTGTAAAA TAGACTTACA TGCAATTtT TGCTGCGACA TTATTGTcAC TTATTCTGG	1500
CAATAAATTa GTTTATTACT GAACtTTTTT TTGGTCAATT TATTACTAGT AACtTTAAAC	1560
TtAAAGAGT GAGATTGTTT GATCAAAAATA AATAAAAATA GAGTGAAGATA GTTGAATCT	1620
GCCATGAAAG CAACACTATA TAGACAAATT ATTtTTATG AAAACACATT TAATAATTG	1680
AGGCTGCAGG AGAATAAGCC ATCGGACACa CCACCAGAAC CATGGCCATG TTGAAAACGA	1740
CGAGTCTTGG GTTCCGGTAA CATTCCCTC TtTAATAATT TCTTATTTTC TGTCAAATA	1800
ATAGTTTT CGAAATTGTA GGCCAGAACG ACCACTGTc AAATTGATT TTAGTGTa	1860
GtAAACAG TTTGCTAGTc TCACAGTTAA CGCGTAATTG ATTCTTTTA ACgATTATA	1920
GAAGTAACAT TTTGTAAAAA TAAATATAc ATTATGGTAT GTGACAACGG ACCACGCTTA	1980
TTTGTATTGG TGAATCTTT ATTACTCCC TCCAATTtT TTAGTGTcA GATTTAGATT	2040
TATGCACATA GATTAATAAA AATATTGTC ACATTTCAA AATAAAAACA CCATTACTTA	2100
TACAACTAAC CATAATTCAA CCAATAAAA TAAATTAGAA AATATTATTtT ATAATTTG	2160
TATTGAAATT ATAAATAAT ACTTATTtTA AAACGAAATT AATTACAAc GACAATTAAA	2220
CTGAAACGGA AAGAAATTAT TAATACtTA TAAAGAGTt TTGAAAGACA	2280
TGTTTATGCG AAACtCATGT GAAAGTCTT GAAATAATAG ATTtGGTAT AAATATTCA	2340
AAATTCTTA AAATAATAAT TATATATTAA TATAATTGT GATAAAATCT CGTCAAAC	2400
TCACTAATGC AAATGCTTT ATTtGAATT TCTTACTCCT CTAATGCAT TTACTTTAT	2460
ACTAATATTa TTtCTTTCT CTAATTGGC GTTcGTAAt AGTTGTCTG TTTTGAAA	2520
ACTAACAAA AATAATAAA ACAAAAGCTT ATAAACACAT AGCATGCAAT GAATATGTAC	2580
GAATATATAc ACCAATAACAT ATCTAAGTAC TATTtTCCa AGTACTTAAT CTGATTACT	2640
AAAATTcATT TTAATTGTTc CTTCAGTTA CCAGAAAGGT TATACAAGAA TTTACCCAC	2700

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agtactcgga tgctcagata cactgtccct ctgccccatgc tcgcttaccc gatctatctg	2760
gtattttta attcctaaaa tttaactacaa gtcatttttag actgtgtttt aaaacaatat	2820
aattatTTTT gtttggtttt actgcagtgg tacagaagtc ctggaaaaga agggtcacat	2880
tttaacccat acagtggttt atttgctcca agcgagagaa agcttattgc aacttcgact	2940
acttgcgtt ccataatgtt ggcaattttt atctgtcttt ctttcctcgt tggccagtc	3000
acagttctca aagtatacgg tggcccttac attgttaagg tcttagtata tcataaaagg	3060
tatataattta ttattcaata tatataactat atgatttgtt tttgtcatat atttttgaaa	3120
tattcagatc ttgtgtatgt gggtggacgc tgcacttac ttgcattacc atggcatg	3180
tgagaagttt ctttggtaca gaggcaaggt aattaaatta actattacaa gtattttaca	3240
aaaaactaat gattgtataat tttgtatata cttaattttt gatgttttgtt gattaataat	3300
aggaatggag ttacttacgt ggaggattaa caactattga tagagattac ggaattttca	3360
acaacattca tcacgacatt ggaactcacc tgatccatca tctttccca caaatccctc	3420
actatcactt ggtcgatgct gtgagtcac tcactctctg gotactttca tcaaaaccat	3480
ttgattaaag ggtgatataat tactaatgtt gtgatTTTAA caaatggaaat gtgacagaca	3540
aaagcagcta aacatgtgtt gggaaagatac tacagagaac caaagacgtc aggagcaata	3600
cgcgcactt tgggtggagag tttggtagca agtattaaaga aagatcatta cgtcagtgac	3660
actggtgaca ttgtttctca cgagactgat c	3691

&lt;210&gt; SEQ ID NO 3

&lt;211&gt; LENGTH: 3829

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Brassica rapa

&lt;400&gt; SEQUENCE: 3

gaacccttc ttcaccacat tccacttccc acactctctt ttttttgaa ttatagagag	60
agaatcctcc tccaaatctc tctctctccc aggtgggtt tggctatggc ccaacgcacc	120
aatgtgaacg gagatgcggg tgcccgaaag gaagaagggtt ttgatecgag cgccacaaccg	180
ccgtttaaga tcggggacat aagggctcgg attcctaagc attgtgggtt gaaaagtcc	240
ttgagatcta ttagatcacgt agccagagac atttgtgcgg tcggggctt ggccattgcc	300
gccgtgtatt ttgatagctg gttctctgt cctctctatt gggtcgccc aggaaccctt	360
ttctgggcca tcttcgttcc cggccacgac tggtaaagg tcttcattt tgcattgcat	420
cgatttattt aatgcacgat ctacgagttt tggttgcgtt ttacttcgtt aatgtattct	480
tttgcgttcc atttttgaa gatctaagat tttttttt agatttctt tttaaatcat	540
tgttccacca ccacccatca tcggtcgtac gactcgttac aacaccacat ctttttttcc	600
tataattact actgtttccg cattttatgg atctctcaac ttataattaa agtataat	660
caagaatatac tattttttt cttaaacaag aaagataata ttgtttctt gttatTTTGG	720
tgtatTTCCA atctatTTCG agatTTGAA atgtgacacg tcattacctt gttgaagtgt	780
ttaaaacaaa catggaaagt ttaaaataat agtgcataaa atgatataat ttttatgtat	840
gaataatgtat gtgaaatata attgaataat ggcagtggac atgggagttt ctcagacatt	900
cctctgtgtt atagtgtggg tggccatatt ctccatttcc tcatcctcgt tccttaccat	960
gggtggtaag tcagtttattt aaccctttt actatattat taattattaa acttgcattt	1020
gtataacttgg tgcaagttgg taaatgtat ctgataactg aaaatctatt cattgctgt	1080

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tctttttttt tttggctaga gacaattta taatcaaata atgcacgtga gaatatgact	1140
atttatgtga ggttagcttt cttattccctg tcgaaaagca tcaaacttt agcaacgaaag	1200
aaaaaaaggaa tcaaattttt tattaaatgc aatgggtcta tgtcttggc attagtttt	1260
tgcataataat ttatattatat tttttctga acagcagcta atttaattat aatcaaataat	1320
tcattttata aataatatta gaccaattat taaaggtagt atattttaag aattattcat	1380
gactttgtt attggaactc cttttatctt ttaatctttt ctatttctcc atttttaataa	1440
atgagaaaact gacttcaaat ctccaataaa gatggctta tgttagtaaca gtataattt	1500
ttgtttggta aatgtAACat catcttcaaa tatcttgaa aatagactta catgcattat	1560
tttgctgcga cattattgtc acttattcct ggcaataat tagtttattta ctgaaaactt	1620
ttttttggc aattttattac tagtaacttt aaacttaaaa gagtgagatt gtttgatcaa	1680
aaaaaaataaa aatagagtga gatagttaga atctgccatg aaagcaacac tatatagaca	1740
attaatttt tatgaaaaca catttaataa tttgaggctg caggagaata agccatcgga	1800
cacaccacca gaaccatggc catgttggaa acgacgagtc ttgggtccg gtaacatttc	1860
cctcttaat aatttctatt tttctgtcaa aataatttagt ttttcgaaat ttgaggccag	1920
aacgaccact tgtcaaattt gatTTTtagc tgttagtaaaa acagtttgct agtgtcacag	1980
ttaaccggta attgattctt tttaacgatt tatagaagta acatTTTgt aaaataaaat	2040
atacattatg gtatgtgaca acggaccacg ctatTTTgtta ttggtaatc tttaattac	2100
tccctccgat ttatTTTgt tgcatTTTgtta gatTTTgtca catagattaa taaaatatt	2160
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ttaaaaacga atttaatTTA caacgacaat taaaactgaaa cgaaaaagaaa ttatTAatAC	2340
ttaattaaag agTTTTtaga aaaattgaaa gacatgttta tgcgaaactc atgtgaaagt	2400
cttTGAATA atagattttg gtataatTTTttcaatTTTttcaatTTTttcaatTTTttcaatTT	2460
ttaatataat ttgtgataaa atctcgtaa aactcaacta atgcAAatgc ttTTTtttgc	2520
aatttcttac ccTCTCTAAAT gcatttactt ttataactaat atttTTTctt ttctcttaatt	2580
tggcatttcg taatagTTTgt tctgtatTTTgt gaaaactaac aaaaataat aaaaacaaaa	2640
gettataaac acatAGCAGTCG caatgaatat gtacgaatat atataccaat acatATCTAA	2700
gtactatTTTttcaatTTTttcaatTTTttcaatTTTttcaatTTTttcaatTTTttcaatTT	2760
gttaccgaa aggttataca agaatttacc ccacagtact cggatgctca gatacactgt	2820
ccctctGCCc atgctcgctt acccgatcta tctggTattt tttaattcctt aaaaattact	2880
acaagtcatt ttagactgtg ttTTTAAACA atataattat ttTTTtttgg ttTTTactgca	2940
gtggTACAGA agtccTGGAA aagaagggtc acatTTTaaac ccatacagtg gtttatttgc	3000
tccaaAGCAG agaaAGCTTt ttgcaacttc gactacttgc tggTCCatTTt tggTGGCAat	3060
tcttatctgt ctTTTCTTCC tcgttggc agtcacagt ctcaaagtat acggcgttcc	3120
ttacattgtt agTTTTCTTAG tatacataa agggtatata ttTattatTC aatataatata	3180
ctatATGATT ttTTTTGTC atatTTTTTt gaaatattca gatTTTGTG atgtggTtgg	3240
acgctgtcac ttacttgcat caccatggc atgatgagaa gttgcTTGG tacagaggca	3300
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taatcttaat tcttgatgtt ttgtgattaa taatAGGAAT ggagttactt acgtggagga	3420
ttaacaacta ttgatagaga ttacggaaatt ttcaacaaca ttcatcacga cattggaaact	3480

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cacgtgatcc atcatcttt cccacaaaatc cctcaactatc acttgggtcga tgctgtgagt    3540
catctcaatc tctggtaact ttcatcaaaa ccatttgatt aaagggtgat taattactaa    3600
tgttagtgatt ttaacaaatg gaatgtgaca gacaaaagca gctaaacatg tggtggaaag    3660
atactacaga gaaccaaaga cgtcaggagc aataccgatc cacttgggtgg agagtttgg    3720
agcaagtatt aagaaagatc attacgtcag tgacactggg gacattgtct tctacgagac    3780
tgatccagat ctctacgttt atgcttctgt caaatcgaaa atcaattaa                      3829
```

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<210> SEQ ID NO 4
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: exon 3, intron 3 border of gene encoding a
      fatty acid desaturase
```

&lt;400&gt; SEQUENCE: 4

```
atgttggaaa cgacgagtct tgggttccgg taatccccct ctcata                      46
```

```
<210> SEQ ID NO 5
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: exon 3, intron 3 border of gene encoding a
      fatty acid desaturase
```

&lt;400&gt; SEQUENCE: 5

```
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```

```
<210> SEQ ID NO 6
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: exon 3, intron 3 border of gene encoding a
      fatty acid desaturase
```

&lt;400&gt; SEQUENCE: 6

```
atgttggaaa cgacgagtct tgggttccgg taatctttcc ctctctcata                      50
```

```
<210> SEQ ID NO 7
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: exon 3, intron 3 border of gene encoding a
      fatty acid desaturase
```

&lt;400&gt; SEQUENCE: 7

```
atgttggaaa cgacgagtct tgggttccgg taacatttcc ctctttaata                      50
```

```
<210> SEQ ID NO 8
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: exon 3, intron 3 border of gene encoding a
      fatty acid desaturase
```

&lt;400&gt; SEQUENCE: 8

```
atgttggaaa cgacgagtct tgggttccag taacatttcc ctctttaata                      50
```

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<210> SEQ ID NO 9  
<211> LENGTH: 497  
<212> TYPE: DNA  
<213> ORGANISM: Brassica napus

<400> SEQUENCE: 9

```

gaccagcgta gcaatgcgaa cggagacgaa aggtttgatc cgagcgcaca accaccgttc      60
aagatcggag atataagggc ggccattctt aagcattgtt gagtaaagag tcctttgaga     120
tccatgagct atgtcgccag agacatttc gccgtcggtt ctcttgcgt cgccgcccgt      180
tattttgata gctggttttt ttggcctttt tattggggcc cccaaggaac cctgttctgg     240
getatctcg tactcggcca cgactggtaa tttttttt ctttcaactt cttaattttt      300
atatgtttat atgtttttt tctgtttttt cattgtttt gatttcttga ccgcacgttc     360
gatatgagat ttcaactgac ttcaagattt gattcttcc aggtttactt ttttcaaatt     420
taattttat tcaccaatt tggccttattt taaaagcaaa aggggatcta agattttaa     480
ttctttgtt tttttt                                         497

```

<210> SEQ ID NO 10  
<211> LENGTH: 349  
<212> TYPE: DNA  
<213> ORGANISM: Brassica napus

<400> SEQUENCE: 10

```

cgtagcaatg tgaacggaga ttccaaggac gaaaggttt atccgagcgc acaaccacccg      60
tttaatcg gagatataag ggctgcgtt cctaaggatt gttgggtcaa gagtcctttg     120
agatccatga gctacgtcgc gagagacatt ttctccgtcg tggctctggc cgccgccc      180
gtgttattttt atagctggtt ctcttagcct ctttatttttgg ccggcccaagg aaccctttc     240
tggccatct tcgtactcgg ccacgactgg taatttaattt ttcaattttt ttttcttca     300
acttcttaat tttgatatgt ttatatgtt tttcgtttt catcggtgt                         349

```

<210> SEQ ID NO 11  
<211> LENGTH: 36  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: portion of fatty acyl-acyl-ACP thioesterase A2 protein

<400> SEQUENCE: 11

Leu	Glu	Asp	Pro	Ala	Gln	Tyr	Ser	Met	Leu	Glu	Leu	Lys	Pro	Arg	Arg
1								10					15		

Ala	Asp	Leu	Asp	Met	Asn	Gln	His	Val	Asn	Asn	Val	Thr	Tyr	Ile	Gly
								20				25		30	

Trp	Val	Leu	Glu												
			35												

<210> SEQ ID NO 12  
<211> LENGTH: 367  
<212> TYPE: PRT  
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 12

Met	Leu	Lys	Leu	Ser	Cys	Asn	Val	Thr	Asp	His	Ile	His	Asn	Leu	Phe
1								10					15		

Ser	Asn	Ser	Arg	Arg	Ile	Phe	Val	Pro	Val	His	Arg	Gln	Thr	Arg	Pro
								20				25		30	

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Ile Ser Cys Phe Gln Leu Lys Lys Glu Pro Leu Arg Ala Ile Leu Ser  
35 40 45

Ala Asp His Gly Asn Ser Ser Val Arg Val Ala Asp Thr Val Ser Gly  
50 55 60

Thr Ser Pro Ala Asp Arg Leu Arg Phe Gly Arg Leu Met Glu Asp Gly  
65 70 75 80

Phe Ser Tyr Lys Glu Lys Phe Ile Val Arg Ser Tyr Glu Val Gly Ile  
85 90 95

Asn Lys Thr Ala Thr Ile Glu Thr Ile Ala Asn Leu Leu Gln Glu Val  
100 105 110

Ala Cys Asn His Val Gln Asn Val Gly Phe Ser Thr Asp Gly Phe Ala  
115 120 125

Thr Thr Leu Thr Met Arg Lys Leu His Leu Ile Trp Val Thr Ala Arg  
130 135 140

Met His Ile Glu Ile Tyr Lys Tyr Pro Ala Trp Ser Asp Val Val Glu  
145 150 155 160

Ile Glu Thr Trp Cys Gln Ser Glu Gly Arg Ile Gly Thr Arg Arg Asp  
165 170 175

Trp Ile Leu Lys Asp Cys Ala Thr Gly Glu Val Ile Gly Arg Ala Thr  
180 185 190

Ser Lys Trp Val Met Met Asn Gln Asp Thr Arg Arg Leu Gln Arg Val  
195 200 205

Thr Asp Glu Val Arg Asp Glu Tyr Leu Val Phe Cys Pro Pro Glu Pro  
210 215 220

Arg Leu Ala Phe Pro Glu Glu Asn Asn Ser Ser Leu Lys Lys Ile Pro  
225 230 235 240

Lys Leu Glu Asp Pro Ala Gln Tyr Ser Met Leu Gly Leu Lys Pro Arg  
245 250 255

Arg Ala Asp Leu Asp Met Asn Gln His Val Asn Asn Val Thr Tyr Ile  
260 265 270

Gly Trp Val Leu Glu Ser Ile Pro Gln Glu Ile Ile Asp Thr His Glu  
275 280 285

Leu Lys Val Ile Thr Leu Asp Tyr Arg Arg Glu Cys Gln Gln Asp Asp  
290 295 300

Ile Val Asp Ser Leu Thr Thr Ser Glu Thr Pro Asn Glu Val Val Ser  
305 310 315 320

Lys Leu Thr Gly Thr Asn Gly Ser Thr Thr Ser Ser Lys Arg Glu His  
325 330 335

Asn Glu Ser His Phe Leu His Ile Leu Arg Leu Ser Glu Asn Gly Gln  
340 345 350

Glu Ile Asn Arg Gly Arg Thr Gln Trp Arg Lys Lys Ser Ser Arg  
355 360 365

&lt;210&gt; SEQ ID NO 13

&lt;211&gt; LENGTH: 223

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Brassica napus

&lt;400&gt; SEQUENCE: 13

Gly Phe Ser Thr Asp Gly Phe Ala Thr Thr Leu Thr Met Arg Lys Leu  
1 5 10 15

His Leu Ile Trp Val Thr Ala Arg Met His Ile Glu Ile Tyr Lys Tyr  
20 25 30

Pro Ala Trp Ser Asp Val Val Glu Ile Glu Thr Trp Cys Gln Ser Glu  
35 40 45

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Gly Arg Ile Gly Thr Arg Arg Asp Trp Ile Leu Arg Asp Ser Ala Thr  
 50 55 60

Asn Glu Val Ile Gly Arg Ala Thr Ser Lys Trp Val Met Met Asn Gln  
 65 70 75 80

Asp Thr Arg Arg Leu Gln Arg Val Thr Asp Glu Val Arg Asp Glu Tyr  
 85 90 95

Leu Val Phe Cys Pro Arg Glu Pro Arg Leu Ala Phe Pro Glu Glu Asn  
 100 105 110

Asn Ser Ser Leu Lys Lys Ile Pro Lys Leu Glu Asp Pro Ala Gln Tyr  
 115 120 125

Ser Met Leu Glu Leu Lys Pro Arg Arg Ala Asp Leu Asp Met Asn Gln  
 130 135 140

His Val Asn Asn Val Thr Tyr Ile Gly Trp Val Leu Glu Ser Ile Pro  
 145 150 155 160

Gln Glu Ile Ile Asp Thr His Glu Leu Gln Val Ile Thr Leu Asp Tyr  
 165 170 175

Arg Arg Glu Cys Gln Gln Asp Asp Ile Val Asp Ser Leu Thr Thr Ser  
 180 185 190

Glu Ile Pro Asp Asp Pro Ile Ser Lys Leu Thr Gly Thr Asn Gly Ser  
 195 200 205

Ala Thr Ser Ser Ile Gln Gly His Asn Glu Ser Gln Phe Leu His  
 210 215 220

<210> SEQ\_ID NO 14  
 <211> LENGTH: 1164  
 <212> TYPE: DNA  
 <213> ORGANISM: Brassica napus

&lt;400&gt; SEQUENCE: 14

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aagtgtggat tctcgacgga tggatttgc acaaactca ccatgaggaa attgcatctc      60
atatgggtca ctgcaagaat gcacatttag atctacaagt acccagcttg gtattttctt     120
ttcttaggct tctttgacta gttgacactt tagaggtcgg agtttgtaaa cctcagagct    180
ttttattact tggtaaacag gagtgtatgtt gttgagatag agacatggtg ccagagtgaa   240
ggaaggattt gaacgagacg tgattggatt ctaaggact ctgctacaaa tgaagttatt   300
gggcgtgcta caaggtttgc caaaaacaga tttgttacta ctattcataa attcatttt   360
ttatctgcct tcaatcaata taataatgca aatcaactgac attagtgcac caacagtaac  420
tccccatatac gttgcttatt tagttataaa gacttatgca tattctggaa cctgagcttg  480
ttttgtttt gaaaaatgtta catgggtctt acagcaagtg ggtgatgtg aaccaagaca  540
caaggccgc tcaaagagtt acagatgaag ttccggacga gtacttggtt ttctgtcctc  600
gagaaccacag gtgaagaaga atcatcatgc ttcccttata attgcttagtt aaacagttaa 660
tatttaagca tggatgttc aacctgttgtt cctctgtatt ttcgttagac tagcgtttcc  720
agaagagaac aatagcagct taaaagaaaat cccaaaacta gaagatccag ctcagtattc 780
tatgcttagag cttaagcttc ggcgagctga tctggacatg aaccagcacg tgaataacgt 840
cacctacatt ggatgggtgc ttgaggttag taccttaata aacgcctacaa aacgtctatc 900
attttaatca tacatatgag ctaactaact attaaatttg agtttggttc cctggtaatg 960
gcagagcata cctcaagaaa tcattgatac gcatgagott caagttataa ctcttagatta 1020
cagaagagaa tgccagcaag atgacattgt agattcactc accacctctg aaatccctga 1080
cgacccgatc tcaaagctta ccgggaccaa cggatctgcc acgtcaagca tacaaggaca 1140
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caatgagagc cagttcttgc atat	1164
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<210> SEQ ID NO 15  
<211> LENGTH: 1163  
<212> TYPE: DNA  
<213> ORGANISM: Brassica napus

<400> SEQUENCE: 15

aagtgtggat tctcgacgga tggatttgcc acaacactca ccatgaggaa attgcacatc	60
atatgggtca ctgcaagaat gcacatttagat atctacaagt acccagctt gatat	120
tctttaggtt tctttacta gttgacactt tagaggtcg agtttgtaaa cctcagagct	180
ttttaatact tggtaaacag gagtgatgtt gttgagatag agacatggtg ccagagtgaa	240
ggaaggattg gaacgagacg tgattggatt ctaagggact ctgctaaaa tgaagttatt	300
gggcgtgcta caagggttc caaaaacaga tttgttacta ctattcataa attcatttt	360
ttatctgcct tcaatcaata taataatgc aatcaactgac attagtcgc caacagtaac	420
tcocatatac gttgttttatt tagttataaa gacttatgca tattctggaa cctgagctt	480
tttttgttg acaaattgtt catgggtt acagcaatg ggtgtatgt aaccaagaca	540
caaggcggct tcaaagagtt acagatgaag ttcccggacga gtacttggtt ttctgtctc	600
gagaaccacg gtgaagaaga gtcatcatgc ttcccttata attgcttagt aaacagttaa	660
tatTTAAGCA TGTGGATCTC AACCTGTTGT TCTCTGTATT TCTCGTAGAC TAGCGTTCC	720
agaagagaac aatagcagct taaagaaaat cccaaaacta gaagatccag ctcagttt	780
tatgcttagag cttaaagctt cgcgagctga tctggacatg aaccagcacg tgaataacgt	840
cacctacatt ggatgggtgc ttgaggttag taccttata aagcctacaa aacgtctatc	900
atTTTAATCA TACATATGAG CTAACTAATC ATAAATTG AGTTGGTTC CCTGGTAATG	960
gcagagcata cctcaagaaa tcattgatac gcatgagctt caagtataa ctctagatta	1020
cagaagagaa tgccagcaag atgacattgt agattcactc accacctctg aaatccctga	1080
cgacccgatc tcaaagctt ccgggaccaa cggatctgcc acgtcaagca tacaaggaca	1140
caatgagagc cagttcttgc ata	1163

<210> SEQ ID NO 16  
<211> LENGTH: 1557  
<212> TYPE: DNA  
<213> ORGANISM: Brassica Napus

<400> SEQUENCE: 16

atgggtggcta cttcgctac gtcgtcggtt tttcatgttc catcttcttc ctgcgttgat	60
actaatggga agggaaacag agtgggtct actaatttg ctggacttaa ctcaacgcca	120
agctctggaa ggatgaaggt taagccaaac gtcaggctc cacccaaatg caacggaaag	180
aaagctaact tgcctggctc tggtagagata tcaaagtctg acaacgagac ttgcacacc	240
gcacacgcac cgaggacgtt tatcaaccag ctacctgact ggagcatgt tcttgcgtcc	300
ataacaacta ttttcttagc ggcggagaaa cagtggatg tgcttgactg gaaacctagg	360
cgttctgata tgattatgga tcctttcggt ttagggagaa tcgttcagga tggcttggt	420
ttccgtcaga attttccat taggtctttagat gagataggct ctgatcgctc tgcgtctata	480
gaaactgtca tgaatcattt acaggtactg ctttgattgt ggttacactc acatgttgc	540
ccaaatagata tatgtctatg acaagctttt atgctaatga caggaaacgg cgcttaatca	600

## US 9,185,861 B2

**59****60**

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tgtgaagtct gccggactgc tggaaaatgg gtttgggtcc actcctgaga tguttaagaa	660
gaatttata tgggtcggtt ctcgtatgc ggttgcgtt gataaaatc ctactggta	720
agccattgtt agtcttagca cttgacttaa aatcatttg catattacag tgtgegtaga	780
tcatttgctt attcaaataat ctgactcaca ggggagatgt tgtgaaagt gatactggg	840
tttagtcgtc tggaaaagaat ggtatgcgtc gtgattggct agttcggat tgcaacttg	900
gagaaaattgt aacgcgagca tcaaggctcg agttcttata ttttggttt ctcagctat	960
tatcgtttg ctctctgtt gtattgttc ctctgccatt agtttataa ttgagtctt	1020
atagttgtat atgtatggca attttcttct ttttgcagt tgtgggtgat gatgaaaaaa	1080
ctcacaagga gattgtcaaa gattcctgaa gaggttcgag gggaaataga gccttattt	1140
gtgaactctg atccctgtcat tgccgaagac agcagaaagt taacaaaact tcatgacaag	1200
actgctgact atgttcgttc tggctctact gtaagttacct taccttcga caagcctgtc	1260
aaaactcttg aggttctaat ggtttgtaa tgaactttt tttggcagcc gaggtggagt	1320
gacttggatg ttaaccagca tggtaacaat gtaaagtaca ttgggtggat actggagat	1380
gctccagcag ggatgctgga gagtcagaag ctgaaaagca tgactctgga gtatgcagg	1440
gagtgccggaa gagacagtgt gcttcgtct ctcaccgcag tctctggatg tcatgtcggt	1500
aacctcggga cagccgggaa agtggagtgt cagcattgc ttgcactcca ggtatgga	1557

&lt;210&gt; SEQ ID NO 17

&lt;211&gt; LENGTH: 1563

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Brassica Napus

&lt;400&gt; SEQUENCE: 17

atgggtggcca cctcagctac atcctcatc ttcccctctcc catcttcccc cctcgacccc	60
accgcaaaaa ccaacaaagt caccacctcc accaacttct ccggcctctc ccccactcca	120
aactcctccg gcaggatgaa ggttaaacc aacgctcagg ccccacccaa gatcaacggc	180
aagagagtcg gtctcccttc tggctcggtg aagcctgata acgagacgtc ctcacagcat	240
cccgccagcac cgaggacgtt catcaaccag ctgcctgact ggagcatgtct tcttgctgca	300
ataacaaccc tcttcttggc ggctgagaag cagtggatg tgcttgactg gaaaccgagg	360
cgtctgacg tgattatgga tccggtttggg tttagggagga tgcgttgcggatggatggatgg	420
ttccgtcaga atttctctat tcggctttat gagataggtg ctgatcgctc tgcgtctata	480
gaaacgggta tgaatcattt acaggacttg attatgatta tgattatgat tgcgttgcgt	540
tgttgtaact ggacaaagtt aatatgtatt gctgttatgg ttatgtatgg aaacggcact	600
caaccatgtt aagactgctg gactgcttgg agatgggttt gggttactc ctgagatgg	660
taagaagaac ttgatttggg ttgttactcg tatgcaggtt gtcgttgcata aatatccatc	720
ttggtaagct attctcaagc aaccctgaga atcaactgtt ctttgcattt ttgttgcattt	780
aaatatctgt ctcacagggg agatgttgc gaagtagata catgggtgag ccagtctgga	840
aagaacggta tgcgtcgtga ttggctatggt cgagatggca atactggaga aattttaca	900
agagcatcaa ggtagatgtt ttatgtatcg gtttaggtatc tggaaatgtt agttactaat	960
gcaaaaatatt atttttgcag tgcgtgggtg atgtatgata aactgacaag aagattatca	1020
aagatccctg aagaggttcg aggggagata gaggcctact ttgttgcattc agacccagtc	1080
cttgcgtgagg acagcagaaa gttactaaa cttgatgaca agactgctga ctatgtcgt	1140
tctggctcta ctgttaagtat gcatactttc tctatgtttc atcaaagcct gtaaaacttct	1200

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gagattctta cagtttat ttggtaattt aaactttgc agccgegttg gagtgacttg	1260
gatgttaacc agcacgttaa caatgtgaag tacatcggtt ggatactgga gagtgcaccc	1320
gtggggatga tggagagtca gaagctgaaa agcatgactc tggagtatcg cagggagtgc	1380
gggaggggaca gtgtgtttca gtccttcacc gcggtttcgg gctgcgtatgt tggttagtctt	1440
gggacagctg gtgaagtggaa atgtcagcac ctgctccgtc tccaggatgg agctgaagt	1500
gtgagaggaa gaacagagtg gagttccaaa acatcaacaa caacttggga cattacaccg	1560
tga	1563

&lt;210&gt; SEQ ID NO 18

&lt;211&gt; LENGTH: 1572

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Brassica Napus

&lt;400&gt; SEQUENCE: 18

atgggtggcca octcagctac atcctcattc ttcccctctcc catcttcccc cctcgacccc	60
accgcaaaaa ocaacaaagt caccacctcc accaacttctt ccggcctcac acccacgccc	120
aactccgcca ggtatgaaggt taaaccaaac gtcaggccc cacccaaatg caacggcaag	180
agagtcggcc tccctggctc ggtggagatc ttgaagectg atagcgagac ttgcacacca	240
gcaccggaga cgttcatcaa ccagctgcct gactggagac tgctccctgc cgccatcag	300
accgttttct tggcggctga gaagcagtgg atgatgctcg actggaaacc gaggegtct	360
gacgtgatta tggatccgtt tgggttaggg aggtatgttcc aggtgggct tggatccgtt	420
cagaattttt ctattcggtc ttatgagata ggtgctgatc gctctgcgtc tatagaaacg	480
gttatgaatc atttacaggt actgattatg attatgatgg tagtcgcttgg ttgttactgg	540
acaaacttaa atatgtattt ctcttatggt tgtgatagga aacggcactc aaccatgtt	600
agactgtgg gctgttggaa gatgggtttt gttctactcc tgagatggg aagaagaact	660
tgatatgggt tgttacttgt atgcagggtt tcgttgataa atatcact tggtaagcta	720
ttctcaaaca actctgagaa tcactgcttc ctttgcgtt catttgcgtt ttcaaataatc	780
tgcctcatag gggagatgtt gtggaaagtag atacatgggt gagccagtct gaaaagaacg	840
gtatgegtcg tgattggctt gttcgggtatc gcaataactgg agagattttt acaagagcat	900
caaggttaga ttttattttt tggttactt gggttagata tctgataatt gagttataat	960
catctccgtg ttgtgtaaac tattttttt gcagtgtgtt ggtgtatgt aataaactga	1020
caagaagatt atcaaagatt cctgaagagg ttgcaggggaa gatagagcct tactttgtt	1080
actcagaccc agtccttgc gaggacagca gaaagttaac aaaacttgc gacaaaactg	1140
ctgtctatgt tcgttctggc tcactgtaa gtacaatac ttcaacttat gttcaacaa	1200
agcctgtaaa tttttgagtc tccttacaggt ttggtaatga actttttgc gccgcgttg	1260
agtgacttgg atgttaacca gcacgttaac aatgtgaagt acatcggtt gatactggag	1320
agtgcgtccag tggggatgtt ggagagtcag aagctgaaa gcatgactt ggagtatgc	1380
agggagtggtt ggagagacag tgcgtccag tccttcaccg cggtttcggg ctgcgtatc	1440
ggtagcctcg ggacagccgg tgaagtggaa tgctcagcatc tgctcagact ccaggatgg	1500
gccgaagtgg tgagaggaag aacagagtgg agttccaaa catcaacaaac aacttggac	1560
atcacaccgt ga	1572

&lt;210&gt; SEQ ID NO 19

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<211> LENGTH: 1664  
<212> TYPE: DNA  
<213> ORGANISM: Brassica Napus

<400> SEQUENCE: 19

atgggtggcta cttccgctac gtcgtcgaaa tttcatgttcc catcttcctc ctctcttgat	60
actaatggga aggggaacag agttgcgtcc acgaacctcg ctggacttaa ctcaacgcca	120
agctctggaa ggatgaaggt taaaccaaacc gtcaggctc cacccaaagat caacgggaag	180
aaagctaact tgccctggttc tgccagagata tcaaagtctg acaacgagac ttgcacaccc	240
gcacccgcac cgaggacgtt tatcaaccag ctgcctgact ggagcatgct tctcgctgcc	300
ataacaacta ttttcttagc ggctgagaaa cagtgatgatc tgcttgactg gaaacccagg	360
cgttctgata tgataatggaa tccttcgggt ttagggagaa tgcgttgcgg tggctttgtg	420
tttcgtcaga atttctccat taggtcttat gagataggtt ctgatcgctc tgctgtata	480
gaaaactgtta tgaatcattt acaggtaggt actacttgc ttgttatcac acttgtcact	540
ggacacccaa tagatata tgctcatgac aagctttat gctaatgaca ggaaacggcc	600
ctaaaccatg tgaagtctgc cggactgctg gaaaatgggt ttggttctac tcccggatg	660
tttaagaaga acttgatatg ggtcggttgc cgtatgcagg ttgtcggttgc taaatatcct	720
acttggtaag ccattgtcag tcttaccact taacttaaaa tcattatgca tattacagtt	780
tgcatacatc attacttattt caaatatctg actaacaggg gagatgttgtt ggaagtggat	840
acatgggtta gtcagtcgg aaagaatggt atgcgtcgat attggctgg tccggatgtc	900
aataactggag aaattgtaac gcgagcatca aggtcagatg tcttattgtt tggttactg	960
actccagcta ttatcatttt gctctctgtt tgtattgtt gctctgcatt taatatgata	1020
atagagactt tatagttgtt tatgtatggc aattttcttc tttttcgtt ttgtgggtga	1080
tgtatgttactt actgacaagg agattgtcaa agattcctga agagggtcg gggaaatag	1140
agccttattt tgcgtactt gatcgtgtca ttgccgaaaga cagcagaaag ttaacaaaac	1200
tggatgacaa gactgtgtac tatgttcgtt cgggtctcac tgtaagtacc ctaccttca	1260
acaaggcttt aaaactcttggt aggttctaat ggttggtaa taaactttt tttcagccga	1320
gttggagtga ctttagatgtt aaccagcatg ttaacaatgt aaagtacatt ggggtggatac	1380
tggagagtgc tccagcagggt atgctggaga gtcagaagct gaaaagcatg actctggagt	1440
atcgcaggaa gtgcgggaga gacagtgtgc ttcaatgtt caccgggtc tctggatgt	1500
atgtcggtaa ctcgggaca gcccgggaaag tggagtgtaa gcatttgctt cgtctccagg	1560
atggagactga agtgggtgaga ggaagaacag ctgaagtggat gagaggaaga acagagtgg	1620
gttccaagat agaagcaaca acttgggaca ctgtacatc gttaa	1664

<210> SEQ ID NO 20  
<211> LENGTH: 1714  
<212> TYPE: DNA  
<213> ORGANISM: Brassica Napus

<400> SEQUENCE: 20

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aatggcaaaa ccaacaaagc caccccccacc aacttctccg gactcaaccc cacaccaaaac	120
tcttcggca ggttaaagggt caaaccaaacc gtcaggctc catccaagat caacggcaag	180
aaagtccttgc tgcaggctc agtacacatc gttaagactg ataataacca cgatctctcg	240
caacaaaacg cacccagaac gttcatcaac cagctacatc actggagcat gcttcgtcc	300

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gccccatcacaa	cggctttctt	agcagctgag	aacgcgtgga	tgtatgcttga	tactaaaccg	360
agacgcgtcg	acatgattat	ggatccgtt	gggttaggga	gaatcggtca	ggatggctt	420
gtgttaccgtc	agaatttcga	tatcagggtct	tatgaaatag	gtgctgatcg	ctctgcacatct	480
atagaaaactg	tcatgaatca	cttacaggta	tattacaatc	acactcggtt	gatactatag	540
cttgaccgc	actgatgtt	gtttttat	ttttataaaat	tgtttagtga	catatagata	600
taggttattt	agatatttct	agggttcc	aacctacc	ggactcaa	cctgtccgt	660
aaatttgagtt	taattttaaa	ccaaaaaaat	ccgataacc	aaaaaaccg	tctgtatcta	720
actcttgtcc	tcatgacagg	aaacggctct	caaccatgt	aagtctgcag	gactgctgg	780
agatgggttt	ggttctacac	ctgagatggt	taagaagaac	ttgatatgg	ttgttactcg	840
tatgcaggtt	gtagttgata	aatatcctac	ttggtaagct	ctcttgccac	ttaacctta	900
acaatatgca	tgaatcattt	gcttattcaa	atgtctgtt	caccagg	gatgttgg	960
aagttagatac	atgggtcgt	aagtctgg	agaatgg	gtgtcg	tggctagtt	1020
gtgattgcaa	tactggagaa	atcttaacac	gcgcata	gttagctt	ttttgtttt	1080
gtttactcca	gctattatct	gattattgag	ttataaccat	ctctatgtt	caaaacagt	1140
tgtgggtgat	gatgaataaa	ctgacaagg	gattatcaa	gottctgaa	gagg	1200
gggaaataga	gccttacttt	gtgaaactcg	acccaaatct	tgccgagg	agcaga	1260
taacaaagct	agatgacaag	actgctgact	atgttcgtc	tggctcacc	gtaa	1320
atattcaact	ctttatctt	tagegtgtaa	aactctt	gagattttat	gagttgg	1380
atgaactttt	gcagecgaga	tggagt	gact	tggatgtt	ccagcat	1440
agtacattgg	ttggatactc	gagagt	gctc	cagtagagat	gatgg	1500
aaagcatgac	tctggagtat	aggagg	gaat	gcgggag	cagtgt	1560
ccgcggtttc	gggatgcgt	gttggtag	cc	tcgggac	tggtga	1620
atttgcttcg	acaccaggat	ggagctgaa	tggtga	agg	acgaac	1680
aaacaccatc	aacaacttgg	gacactacat	cgta			1714

&lt;210&gt; SEQ ID NO 21

&lt;211&gt; LENGTH: 1891

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Brassica Napus

&lt;400&gt; SEQUENCE: 21

atgggtggcca	cctctgctac	atcctcattc	ttccctctcc	catcttcc	tctcgac	60
aatggcaaaa	ccaacaaact	cacccccc	aacttctctg	gactcaac	cataccaa	120
tcttccggca	ggttaaagg	caaacc	ac	gcccaag	ctc	180
aatgtctctt	tgccagg	gtc	agtacacatc	gtaaagact	ataataac	240
caacaaacacg	caccc	caac	gttcatca	cag	ctac	300
gccccatcacaa	cggctttctt	agctgctgag	aaacagt	ggta	ctcgaa	360
aggcg	ttctgat	ttat	ggatccgtt	gggttagg	ggatcg	420
gtgttaccgtc	agaacttcga	tatcagg	tatgaaat	gtgctgat	ctctgcgt	480
atagaaacag	tcatgaacca	cttacagg	tattacaatc	acactcg	att	540
cttgacatgt	tgg	ttt	tat	attgtt	atg	600
tataggtt	at	ttat	ctaggtt	c	acaac	660

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gaaatttata atattaatac cgaacagagt tttatTTAA accaaaaaat cagttgaccc	720
gcacgggatg ttggTTTTA tctatTTTAc acattgttta aggacattt taaacatata	780
aatataggTT atttagatAT ttcttaggttc ctacgaacct acccgaaat ttataatacc	840
cgaacatagt ttaatttta aaccaaaaaa tccaataccc gaaaaaacca atctgtgata	900
tgcatgatct aactcttgTC ctcgtgacag gaaacggcTC tcaaccatgt gaagtctgCT	960
ggactgctgg gagatggTT tggTTCTacc cctgagatgg ttaagaagaa cttgatATgg	1020
gtcgTTactc gtatgcaggT tgcgttGat aaatatccta ctggtaAGC cctcttagca	1080
cttaacCTTA aaacaataATG catgaatcat ttgCTTattc aaatgtCTG tcaccaggG	1140
gagatgttGT tgaagtAGat acatggGta gtaagtctGG gaagartggT atgcgtcGT	1200
attggCTTGT tcgggATTGT aatactggAG aaattttaAC aagAGcatCA aggttagCTT	1260
cttttGTTT actccagCTA ttatctgatt attgagttat aaccatctcT gtgttgcAAA	1320
acagtgtgtG ggtgtatgtG aataaAGtGA caaggAGatt atcaaAGcTT cctgaAGagg	1380
ttcgaggGGA aatagAGcCT tacttTGta actctgacCC tATCCTGCC gaggacAGca	1440
gaaaGttaAC aaaACTAGat gagaAGactG ctgactatgt tgcgtetGGt ctcaccGtaA	1500
gtataaATAt ttgttttAt ctTCAGCAA gtgagattct gatgggTTG gtgattatCT	1560
aactttGca gccgagatGG agtGacttGG atgttaACca gcatgttAAc aacGtGAAGt	1620
acattggTTG gatactcGAG agtGctCCAG tggagatGAT ggagaAGcat aagctgaaaa	1680
gcatgactCT ggatGatAGG agggAAatGCG ggagAGacG tGtGCTTCAG tCTCTCACCG	1740
cggTTTcGGG ttGcgtGTT ggtGcCTG ggacAGctGG tgaAGTggAA tGtCAGcATT	1800
tgcTTcGACT ccaggatGGA gctGAAGTGG tgaAGGAGcG aacagtGTGG agttccAAA	1860
caccatcaAC aacttggGAC actacatcGT a	1891

&lt;210&gt; SEQ\_ID NO 22

&lt;211&gt; LENGTH: 1557

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Brassica Napus

&lt;400&gt; SEQUENCE: 22

atggTggCTA cttGcgCTAC gtcgtcGTT tttcatgttC catcttCTC ctcgttGAT	60
actaatGGGA aGGGGAACAG agtGggGTCT actaatttG CTGGACTTAA ctcaacGCCA	120
agctCTGGGA ggatGAAGGT taAGCCAAAC gcttagGTC cacCCAAAGAT caacGGGAAG	180
aaAGCTAAct tgcCTGGCTC tGtagAGATA tcaaAGtCTG acaACGAGAC ttGcGAACCC	240
geacacGcac CGAGGACGTT tatcaACCAG ctacCTGACT ggAGCATGCT tcttGCTGCC	300
ataacaACTA ttttCTTAGC GGCggAGAAA cAGTGGATGA tgcttGACTG gaaACCTAGG	360
cgttCTGATA tgattATGGA tccTTTcGGT ttagGGAGAA tGtttCAGGA tggTCTTGT	420
tTCCGTCAGA atTTTCCAT tagtGTTTAT gagatAGGTG ctGatGcTC tGcGTCTATA	480
gaaACTGTCA tgaatCATT acAGGTACTG ctttGATTGt ggttACACTC acatGTTGTC	540
ccaaTAGATA tatGCTCATG acaAGCTTT atGCTAATGA cAGGAAACGG CGCTTAATCA	600
tGtGAAGTCT GCGGACTGC tggAAAATGG GTTGGGTCC ACTCCTGAGA tGTTTAAGAA	660
gaatttGATA tggGTCGTT ctcgtatGCA ggttGTCGTT gataAAATAC ctacttGGA	720
agccATTGTT agtCTTAGCA cttGACTTAA aatCATTtG catattACAG tGtGeGTAGA	780
tcatttGCTT attCAAATAT ctGACTCACA gggGAGATGT tGtGGAAGTG gataACTTGGG	840
ttagTCAGTC tggAAAGAAT ggtatGCGTC gtGATTGGCT agttcGGGAT tgcaataCTG	900

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gagaaaattgt aacgcgagca tcaaggtag agttcttata tttgggtta ctccagctat 960  
 tatcgtttg ctctctgttt gtattgttc ctctgccatt agtttgataa ttgagtcttt 1020  
 atagttgtat atgtatggca atttcttct tttgcagtt tgtgggtat gatgaataaa 1080  
 ctcacaagga gattgtcaa gattcctgaa gaggttcgag gggaaataga gccttatttt 1140  
 gtgaactctg atcctgtcat tgccgaagac agcagaaagt taacaaaact tgatgacaag 1200  
 actgctgact atggtcggtc tggctcaact gtaagtgact taccttcga caagectgtc 1260  
 aaaactttt aggttctaat gggttggtaa tgaactttt tttggcagcc gaggtggagt 1320  
 gacttggatg ttaaccagca tggtaacaat gtaaagtaca ttgggtggat actggaggt 1380  
 getccagcag ggatgctgga gagtcagaag ctgaaaagca tgactctgga gtatgcagg 1440  
 gagtgccggga gagacagtgt gcttcagttc ttcaccgcag tctctggatg tgatgtcggt 1500  
 aacctcggga cagccgggaa agtggagtgt cagcatttgc ttgcactcca ggatgga 1557

&lt;210&gt; SEQ ID NO 23

&lt;211&gt; LENGTH: 1572

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Brassica Napus

&lt;400&gt; SEQUENCE: 23

atgggtggcca cctcagctac atcctcatc ttcctctcc catcttcccc cctcgacccc 60  
 accgcaaaaa ccaacaaagt caccacctcc accaacttct ccggcctcac acccacggcg 120  
 aactcggcca ggatgaaggt taaaccaaac gctcaggccc cacccaaatg caacggcaag 180  
 agagteggcc tccctggctc ggtggagatc ttgaagctg atagcgagac ttgcacca 240  
 gcaccggagga cgttcatcaa ccagctgcct gactggagca tgctccctgc cgccatcag 300  
 accgtttct tggcggctga gaagcagtgg atgatgctcg actggaaacc gaggegtct 360  
 gacgtgatta tggatccgtt tgggttaggg aggatcgatc aggatggct tggatccgt 420  
 cagaatttt ctattcggtc ttatgagata ggtgctgatc gctctgcgtc tatagaacg 480  
 gttatgaatc attacaggt actgattatg attatgatg tagtcgctg ttgttactgg 540  
 acaaacttaa atatgtattt ctcttatgt tggatagga aacggcactc aaccatgtta 600  
 agactgtgg gctgttgtt gatgggttt gttctactcc tgagatgggt aagaagaact 660  
 tgatatgggt tggatccgtt atgtaggtt tggatggatc atatctact tggatggat 720  
 ttctcaaaca actctgagaa tcactgcctc ctttgcgtt catttgccta ttcaaatac 780  
 tgcctcatag gggagatgtt gtggaaatgtt atacatgggt gagccagtc gaaagaacg 840  
 gtatgcgtcg tgatggctt gttcggatg gcaatactgg agagatggta acaagagcat 900  
 caaggtaga ttttattttt tggttactt gggtttagata tctgataatt gagttataat 960  
 catctccgtt tggatggatc tattttttt gcagttgtgtt ggtgtatgtt aataaactgt 1020  
 caagaagatt atcaaagatt cctgaagagg ttgcaggggaa gatagagcct tactttgtta 1080  
 actcagaccc agtccttgcc gaggacagca gaaagtttac aaaacttgc gacaaaactg 1140  
 ctgtctatgt tggatggatc tctactgtt gatcaaaatc ttcaactatc gttcaacaa 1200  
 agcctgtaaa tttttgttgc tcttacaggat ttgttgcactt ggcgcgttgg 1260  
 agtgacttgg atgttaacca gcacgttac aatgtgaagt acatcggtt gataactggag 1320  
 agtgctccag tggggatgtt ggagagtcgaa aagctgaaaa gcatgactt ggtatcgc 1380  
 agggagtggtt ggagagacag tggatccgtt tccctcaccg cggttcggg ctgcgtatc 1440

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ggtagcctcg ggacagccgg tgaagtggaa tgcagcatc tgctcagact ccaggatgga	1500
gccgaagtgg tgagaggaag aacagagtgg agttccaaa catcaacaac aacttgggac	1560
atcacaccgt ga	1572

<210> SEQ ID NO 24  
<211> LENGTH: 1500  
<212> TYPE: DNA  
<213> ORGANISM: Brassica Napus

<400> SEQUENCE: 24

atgggtggcca cctcagctac atcctcattc ttccctctcc catcttcccc cctcgacccc	60
accgcaaaaa ccaacaaagt caccacctcc accaacttct ccggcctcac acccacgccc	120
aactccgcca ggtatgaaggt taaaccaaac gtcagggccc cacccaaagat caacggcaag	180
agagtcggcc tccctggctc ggtggagatc ttgaagcctg atagcgagac ttgcacca	240
gcaccgagga cgttcatcaa ccagctgcct gactgaagca tgctcctcgc cgccatcact	300
accgtcttct tggcggtctga gaagcagtgg atgatgctcg actggaaacc gaggcggtct	360
gacgtgatta tggatccgtt tgggttaggg aggatcggtc aggtatggct tggatccgt	420
cagaattttt ctattcggtc ttatgagata ggtgctgatc gctctgcgtc tatagaaacg	480
gttatgaatc atttacaggt actgattatg attatgatg tagtcgcttg ttgttactgg	540
acaaacttaa atatgtattt ctcttatgt tggatagga aacggcactc aaccatgtta	600
agactgctgg gctgcttgg gatgggtttt gttctactcc tgagatgggtt aagaagaact	660
tgatatgggt tggatccgtt atgcagggtt tcgttgataaa atatctact tggatagct	720
ttctcaaaca actctgagaa tcactgcttc ctttgtgagt catttgctta ttcaaataatc	780
tgccctcatag gggagatgtt gtggaaagtag atacatgggtt gagccagtc ggaaagaacg	840
gtatgcgtcg tgattggctt gttcgggatg gcaatactgg agagatttt acaagagcat	900
caagggtttaga ttttattttt tggttactt gggtagata tctgataatt gagttataat	960
catctccgtg ttgtgtaaac tattttttt gcagtgtgtt ggtgtatgtt aataaactgtt	1020
caagaagatt atcaaagatt cctgaagagg ttcgaggggatg gatagagcct tactttgtt	1080
actcagaccc agtccttgc gaggacagca gaaagtttac aaaacttgc gacaaaactg	1140
ctgtctatgt tcgttctggc ctcactgtaa gtacaaatac ttcaactctat gttcaacaa	1200
agcctgtaaa tttttagtc tcttacaggt ttggtaatgtt accttttgc gccgcgttgg	1260
agtgacttgg atgttaacca gcacgttac aatgtgaagt acatcggtt gataactggag	1320
agtgcgtccag tggggatgtt ggagagtcag aagctgaaaa gcatgactct ggagtatcgc	1380
aggggagtgtt ggagagacag tggatcccg tccctcaccc cgggttccgg ctgcgtatc	1440
ggtagcctcg ggacagccgg tgaagtggaa tgcagcatc tgctcagact ccaggatgga	1500

<210> SEQ ID NO 25  
<211> LENGTH: 1664  
<212> TYPE: DNA  
<213> ORGANISM: Brassica Napus

<400> SEQUENCE: 25

atgggtggcta cttccgctac gtcgtcggtt ttcatgttcc catcttccctc ctctcttgc	60
actaatggaa agggggaaacag agttgcgtcc acgaacttgc ctggactttaa ctcaacgcca	120
agctctggaa ggtatgaaggt taaaccaaac gtcagggccc cacccaaagat caacggcaag	180
aaagcttaact tgcctggctt tgcagagata tcaaaatgtt acaacgagac ttgcacca	240

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gcacccgcac cgaggacgtt tatcaaccag ctgcctgact ggagcatgct tctcgctgcc	300
ataacaacta ttttcttagc ggctgagaaa cagtgaatga tgcttgactg gaaaccagg	360
cgttctgata tgataatggc tccttcggt ttagggagaa tcgttcagga tggcttgtg	420
tttcgtcaga atttctccat taggtcttat gagataggtg ctgatcgctc tgcgtctata	480
gaaaactgtta tgaatcattt acaggttagt actacttga ttgttatcac acttgtca	540
ggacacccaa tagatata tgctcatgac aagctcttat gctaatgaca ggaaacggcc	600
ctaaaccatg tgaagtctgc cggactgtg gaaaatgggt ttggttctac tcccagatg	660
ttaagaaga acttgatatg ggtcggtgt cgtatgcagg ttgtcggtga taaatatcct	720
acttggtaag ccattgtcag tcttaccact taacttaaaa tcattatgca tattacagtt	780
tgcatacatc attacttatt caaatatctg actaacaggg gagatgttgtt ggaagtggat	840
acatgggtta gtcagtcgg aaagaatggt atgcgtcggtt attggctggt tcgggatgc	900
aataactggag aaattgtAAC gcgagcatca aggtcagagt tcttattgtt tggttactg	960
actccagcta ttatcatttt gctctctgtt tgtattgttt gctctgcacat taatatgata	1020
atagagactt tataatgtt tatgtatggc aattttcttc tttttgcagt ttgtgggtga	1080
tgtatgataa actgacaagg agattgtcaa agattcctga agaggttgtt gggaaatag	1140
agccttattt tgtgaactct gatcctgtca ttgccgaaga cagcagaag ttaacaaaac	1200
tggatgacaa gactgctgac tatgttcgtt cgggtctcac tgtaagtacc ctacattca	1260
acaagecttt aaaactctt aggttctaat ggtttgtaa taaactttt tttcagccga	1320
gttggagtga ctttagatgtt aaccagcatg ttaacaatgt aaagtacatt gggtgatgc	1380
tggagagtgc tccagcaggg atgctggaga gtcagaagct gaaaagcatg actctggagt	1440
atcgcaggga gtgcgggaga gacagtgtgc ttcaagtctc caccgggtc tctggatgt	1500
atgtcggtaa ctcgggaca gccccggaa tggagtgtca gcatttgctt cgtctccagg	1560
atggagctga agtgggtgaga ggaagaacag ctgaagtggt gagaggaaga acagagtgg	1620
gttccaagat agaagcaaca acttggaca ctgctacatc gtAA	1664

&lt;210&gt; SEQ ID NO 26

&lt;211&gt; LENGTH: 5

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: amino acid motif

&lt;400&gt; SEQUENCE: 26

His	Glu	Cys	Gly	His
1				5

&lt;210&gt; SEQ ID NO 27

&lt;211&gt; LENGTH: 6

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: amino acid motif

&lt;400&gt; SEQUENCE: 27

Lys	Tyr	Leu	Asn	Asn	Pro
1					5

&lt;210&gt; SEQ ID NO 28

&lt;211&gt; LENGTH: 10

&lt;212&gt; TYPE: PRT

-continued

<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: amino acid motif  
  
<400> SEQUENCE: 28  
  
Asp Arg Asp Tyr Gly Ile Leu Asn Lys Val  
1 5 10  
  
<210> SEQ\_ID\_NO 29  
<211> LENGTH: 369  
<212> TYPE: PRT  
<213> ORGANISM: Brassica napus  
  
<400> SEQUENCE: 29  
  
Met Val Val Ala Met Asp Gln Arg Thr Asn Val Asn Gly Asp Ala Gly  
1 5 10 15  
  
Ala Arg Lys Glu Glu Gly Phe Asp Pro Ser Ala Gln Pro Pro Phe Lys  
20 25 30  
  
Ile Gly Asp Ile Arg Ala Ala Ile Pro Lys His Cys Trp Val Lys Ser  
35 40 45  
  
Pro Leu Arg Ser Met Ser Tyr Val Ala Arg Asp Ile Cys Ala Val Ala  
50 55 60  
  
Ala Leu Ala Ile Ala Ala Val Tyr Phe Asp Ser Trp Phe Leu Cys Pro  
65 70 75 80  
  
Leu Tyr Trp Val Ala Gln Gly Thr Leu Phe Trp Ala Ile Phe Val Leu  
85 90 95  
  
Gly His Asp Cys Gly His Gly Ser Phe Ser Asp Ile Pro Leu Leu Asn  
100 105 110  
  
Ser Val Val Gly His Ile Leu His Ser Phe Ile Leu Val Pro Tyr His  
115 120 125  
  
Gly Trp Arg Ile Ser His Arg Thr His His Gln Asn His Gly His Val  
130 135 140  
  
Glu Asn Asp Glu Ser Trp Val Pro Leu Pro Glu Arg Leu Tyr Lys Asn  
145 150 155 160  
  
Leu Pro His Ser Thr Arg Met Leu Arg Tyr Thr Val Pro Leu Pro Met  
165 170 175  
  
Leu Ala Tyr Pro Ile Tyr Leu Trp Tyr Arg Ser Pro Gly Lys Glu Gly  
180 185 190  
  
Ser His Phe Asn Pro Tyr Ser Gly Leu Phe Ala Pro Ser Glu Arg Lys  
195 200 205  
  
Leu Ile Ala Thr Ser Thr Thr Cys Trp Ser Ile Met Leu Ala Ile Leu  
210 215 220  
  
Ile Cys Leu Ser Phe Leu Val Gly Pro Val Thr Val Leu Lys Val Tyr  
225 230 235 240  
  
Gly Val Pro Tyr Ile Ile Phe Val Met Trp Leu Asp Ala Val Thr Tyr  
245 250 255  
  
Leu His His His Gly His Asp Glu Lys Leu Pro Trp Tyr Arg Gly Lys  
260 265 270  
  
Glu Trp Ser Tyr Leu Arg Gly Gly Leu Thr Thr Ile Asp Arg Asp Tyr  
275 280 285  
  
Gly Ile Phe Asn Asn Ile His His Asp Ile Gly Thr His Val Ile His  
290 295 300  
  
His Leu Phe Pro Gln Ile Pro His Tyr His Leu Val Asp Ala Thr Lys  
305 310 315 320  
  
Ala Ala Lys His Val Leu Gly Arg Tyr Tyr Arg Glu Pro Lys Thr Ser  
325 330 335

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Gly Ala Ile Pro Ile His Leu Val Glu Ser Leu Val Ala Ser Ile Lys  
 340 345 350

Lys Asp His Tyr Val Ser Asp Thr Gly Asp Ile Val Phe Tyr Glu Thr  
 355 360 365

Asp

<210> SEQ\_ID NO 30

<211> LENGTH: 383

<212> TYPE: PRT

<213> ORGANISM: Brassica rapa

<400> SEQUENCE: 30

Met Val Val Ala Met Asp Gln Arg Thr Asn Val Asn Gly Asp Ala Gly  
 1 5 10 15

Ala Arg Lys Glu Glu Gly Phe Asp Pro Ser Ala Gln Pro Pro Phe Lys  
 20 25 30

Ile Gly Asp Ile Arg Ala Ala Ile Pro Lys His Cys Trp Val Lys Ser  
 35 40 45

Pro Leu Arg Ser Met Ser Tyr Val Ala Arg Asp Ile Cys Ala Val Ala  
 50 55 60

Ala Leu Ala Ile Ala Ala Val Tyr Phe Asp Ser Trp Phe Leu Cys Pro  
 65 70 75 80

Leu Tyr Trp Val Ala Gln Gly Thr Leu Phe Trp Ala Ile Phe Val Leu  
 85 90 95

Gly His Asp Cys Gly His Gly Ser Phe Ser Asp Ile Pro Leu Leu Asn  
 100 105 110

Ser Val Val Gly His Ile Leu His Ser Phe Ile Leu Val Pro Tyr His  
 115 120 125

Gly Trp Arg Ile Ser His Arg Thr His His Gln Asn His Gly His Val  
 130 135 140

Glu Asn Asp Glu Ser Trp Val Pro Leu Pro Glu Arg Leu Tyr Lys Asn  
 145 150 155 160

Leu Pro His Ser Thr Arg Met Leu Arg Tyr Thr Val Pro Leu Pro Met  
 165 170 175

Leu Ala Tyr Pro Ile Tyr Leu Trp Tyr Arg Ser Pro Gly Lys Glu Gly  
 180 185 190

Ser His Phe Asn Pro Tyr Ser Gly Leu Phe Ala Pro Ser Glu Arg Lys  
 195 200 205

Leu Ile Ala Thr Ser Thr Cys Trp Ser Ile Met Leu Ala Ile Leu  
 210 215 220

Ile Cys Leu Ser Phe Leu Val Gly Pro Val Thr Val Leu Lys Val Tyr  
 225 230 235 240

Gly Val Pro Tyr Ile Ile Phe Val Met Trp Leu Asp Ala Val Thr Tyr  
 245 250 255

Leu His His Gly His Asp Glu Lys Leu Pro Trp Tyr Arg Gly Lys  
 260 265 270

Glu Trp Ser Tyr Leu Arg Gly Leu Thr Thr Ile Asp Arg Asp Tyr  
 275 280 285

Gly Ile Phe Asn Asn Ile His His Asp Ile Gly Thr His Val Ile His  
 290 295 300

His Leu Phe Pro Gln Ile Pro His Tyr His Leu Val Asp Ala Thr Lys  
 305 310 315 320

Ala Ala Lys His Val Leu Gly Arg Tyr Tyr Arg Glu Pro Lys Thr Ser  
 325 330 335

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Gly Ala Ile Pro Ile His Leu Val Glu Ser Leu Val Ala Ser Ile Lys  
 340 345 350

Lys Asp His Tyr Val Ser Asp Thr Gly Asp Ile Val Phe Tyr Glu Thr  
 355 360 365

Asp Pro Asp Leu Tyr Val Tyr Ala Ser Val Lys Ser Lys Ile Asn  
 370 375 380

<210> SEQ ID NO 31

<211> LENGTH: 386

<212> TYPE: PRT

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 31

Met Val Val Ala Met Asp Gln Arg Thr Asn Val Asn Gly Asp Pro Gly  
 1 5 10 15

Ala Gly Asp Arg Lys Lys Glu Glu Arg Phe Asp Pro Ser Ala Gln Pro  
 20 25 30

Pro Phe Lys Ile Gly Asp Ile Arg Ala Ala Ile Pro Lys His Cys Trp  
 35 40 45

Val Lys Ser Pro Leu Arg Ser Met Ser Tyr Val Val Arg Asp Ile Ile  
 50 55 60

Ala Val Ala Ala Leu Ala Ile Ala Val Tyr Val Asp Ser Trp Phe  
 65 70 75 80

Leu Trp Pro Leu Tyr Trp Ala Ala Gln Gly Thr Leu Phe Trp Ala Ile  
 85 90 95

Phe Val Leu Gly His Asp Cys Gly His Gly Ser Phe Ser Asp Ile Pro  
 100 105 110

Leu Leu Asn Ser Val Val Gly His Ile Leu His Ser Phe Ile Leu Val  
 115 120 125

Pro Tyr His Gly Trp Arg Ile Ser His Arg Thr His His Gln Asn His  
 130 135 140

Gly His Val Glu Asn Asp Glu Ser Trp Val Pro Leu Pro Glu Arg Val  
 145 150 155 160

Tyr Lys Lys Leu Pro His Ser Thr Arg Met Leu Arg Tyr Thr Val Pro  
 165 170 175

Leu Pro Met Leu Ala Tyr Pro Leu Tyr Leu Cys Tyr Arg Ser Pro Gly  
 180 185 190

Lys Glu Gly Ser His Phe Asn Pro Tyr Ser Ser Leu Phe Ala Pro Ser  
 195 200 205

Glu Arg Lys Leu Ile Ala Thr Ser Thr Cys Trp Ser Ile Met Phe  
 210 215 220

Val Ser Leu Ile Ala Leu Ser Phe Val Phe Gly Pro Leu Ala Val Leu  
 225 230 235 240

Lys Val Tyr Gly Val Pro Tyr Ile Ile Phe Val Met Trp Leu Asp Ala  
 245 250 255

Val Thr Tyr Leu His His His Gly His Asp Glu Lys Leu Pro Trp Tyr  
 260 265 270

Arg Gly Lys Glu Trp Ser Tyr Leu Arg Gly Gly Leu Thr Thr Ile Asp  
 275 280 285

Arg Asp Tyr Gly Ile Phe Asn Asn Ile His His Asp Ile Gly Thr His  
 290 295 300

Val Ile His His Leu Phe Pro Gln Ile Pro His Tyr His Leu Val Asp  
 305 310 315 320

Ala Thr Lys Ala Ala Lys His Val Leu Gly Arg Tyr Tyr Arg Glu Pro

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325	330	335
Lys Thr Ser Gly Ala Ile Pro Ile His Leu Val Glu Ser Leu Val Ala		
340	345	350
Ser Ile Lys Lys Asp His Tyr Val Ser Asp Thr Gly Asp Ile Val Phe		
355	360	365
Tyr Glu Thr Asp Pro Asp Leu Tyr Val Tyr Ala Ser Asp Lys Ser Lys		
370	375	380
Ile Asn		
385		
<210> SEQ ID NO 32		
<211> LENGTH: 4676		
<212> TYPE: DNA		
<213> ORGANISM: Brassica napus		
<400> SEQUENCE: 32		
aaacgtaaac aatttatacg accacagttc gaaaataaaa acaatttata cgaccagaaa	60	
tggcaaaatg ttgttcttag cattttttt ttaactttac ttttgcgtaa aacacatttc	120	
tccaaattgg tttcattgcg ttgaacgacg taacaaagta atacacactaa ccctttttt	180	
tggaacatta tacacccaac ccattgtaca aaagttacag ctaaattacc ctttttattc	240	
ttttgataaa taaaaaaaata aatttataat cattaaaaaa taatttggag tattttctca	300	
atgtccatat atacatcttc tcccttata taagccaacc tcacacaccc aaaaaatcca	360	
tcaaaccctt cttcaccaca tttcactgaa aggccacaca tctagagaga gaaacttcgt	420	
ccaaatctct ctctccagca atgggttgtt ctagggacca gcgcagcaat gttaacggag	480	
attccggtgc ccggaggaa gaagggtttg atccaagcga acaaccaccc tttaaagatcg	540	
gagatatcg ggccggcatt cctaagcatt gttgttttag gtttaattct tttgaggtta	600	
ccttttcatg ttcattattt aaaaaataaa aataaaatatt aggtatctaag attttttct	660	
tcatcagttc aagcatcatc actcatcagt cgtaaagactc gtaacaaaat atcttcttt	720	
ctataattaa tattatttcc gcatttaatg gatctacgtt ttgatgttct caaattttgt	780	
ttctctttct ctagatcccc ggaactttta attataatta tagtataagta taatataaag	840	
aaaaataact gtttattttt tttggcaaca aatataattac tcttgggttct ttgacaagaa	900	
aaaaatataat tttttttttt cttctttttg ttttccaatc tattttcgag atttagacaa	960	
gtgacacgtc atataccgga tttgttacct tttttaagag tttgggttaa aacaaatgt	1020	
aaaaagttaa aataaaattgt gcaataaaatg ataaatacgt ttttatgtt aacaatgtg	1080	
tgaaaataaa attgaataat ggcagtggac atgggagttt ttcagacatt cctctgtga	1140	
acagtgtgg tggcacatt cttcattcat tcatcctcg tttttttttt gtttgtaag	1200	
tcatattttt actatttcca tggtaactat tagtacttgtt ttgttattttt cttacatttt	1260	
cgtttgcatt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt	1320	
ccaaactgttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt	1380	
aatgcgtgtg acaaatatgag gttgttttc tttttttttt tttttttttt tttttttttt	1440	
aaaagaatcc aaaactttttt tttttttttt tttttttttt tttttttttt tttttttttt	1500	
gtttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt	1560	
gtttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt	1620	
gaggattttt agttttttttt ttactttttt ccgacacaaat gtttagtagt aaaaagcatt	1680	
aaatgtttttt ttgtcaaaa aaaaaagaat gggattgtta gagcactcta ttgttagtt	1740	

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ttcaataaat ataccaacta aaaaaacaaa ataaatataa aatgagttag attgttaat	1800
cattatagag acaatttcat ttccacaaaa ataaataat acataactt ttataattgg	1860
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cgagtcttgg gttccggtaa tcttcctac tctcgttagt tctcttgct tttatttatt	1980
tgttgtttt tcggaattta ttcttatgtc tatgttctta ggattctata tgtttatttt	2040
attagtttat gtttcagtc tgaggtcaga ccgaccactt gtcagatctg tttctagct	2100
gtagtaaaaa acaatttgc agtgtatag tttagcataa ttgatcttg tagagcattt	2160
ccaaaacaaa ctttataatt ttaaatatac agtttttg tctctaaaaa agaatttaaa	2220
aattttaaag ttgagggac gaaacttcaa atttgaactt tcactactca acttcaaatt	2280
tgaatttca tctttttat ttacattttg atcattataa ttaattatac attacattta	2340
tgattcttaa gtatcccattt atttattgtt ttaattctta aattttttat acatcataaa	2400
tatttccaaat ttgtttttat aaattcaaat ttacacaaaa aaagtaataa aaattttaaa	2460
taagatttt aatattttaa aactataattt aggcaaaaaa aatattacaa aaaaatgtaa	2520
taaaaaactt aaaaataagat atatcaagac ataattatta gaaattttaa atattataac	2580
aatattataa atctgtaaa ttgtctcaa aacctcaaaa atttctaaat tattgtccaa	2640
acaaatttgc ttaaccgaat atggggcatt aaaaaataa ttttatggaa tagtgtggta	2700
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caaaaacatc attaattatt tacctaacc caattcaacc aatataaaaa tagaagatat	3180
attaccattt gtcatacaac attaattattt aataaattttt acatagaaaa ccgaaaacga	3240
catataattt ggaacaaaaa aatttctcta aaacgactta tattaaaaaa cggagggagt	3300
agtacctaac tttaacgatg gaccacttattt attcgagtcc ttagcataaa atgattctcc	3360
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aattttaaa aagaaatgta cagaaagcaa taatggtag tattgattaa tcttaattt	4260
tgtatgtttt catacaataa taggaatgga gttatattacg tggaggatata acaactattg	4320
atagagatta cggaaatcttc aacaacatcc atcacgacat tggaaactcac gtgatccatc	4380
atctttccc acaaattccct cactatcact tggtcgatgc ggtgagtgtat ctatcttct	4440
ctctctctag tttcatttga ttaaatggtag attaattact aatttaattta atgaatttgt	4500
gacagacgag agcagctaa catgtgttag gaagatacta cagagagccg aagacgttag	4560
gagcaataacc gattcacttg gtggagagtt tggtcgcaag tattaaaaaa gatcattacg	4620
tcaagtacac ttgtgtatatt gtcttctacg agacagatcc agatctctac gtttat	4676

&lt;210&gt; SEQ ID NO 33

&lt;211&gt; LENGTH: 4935

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Brassica napus

&lt;400&gt; SEQUENCE: 33

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ttgaaacatta tacacccaaac ccattgtaca aaagttacag ctaaatttacc ctttttattc	240
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atgtccatat atacatcttc tccctttata taagccaacc tcacacaccc aaaaaatcca	360
tcaaacccttt ttccaccaca ttctactgaa aggccacaca tctagagaga gaaacttcgt	420
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gagatatacg ggcggcgatt cctaagcatt gttgggtgaa gagtccttgc agatctatga	600
gtacgtcgc cagagacatt ttgcggcgtcg cggctctggc catggccgccc gtgtattttgc	660
atagctggtt ctctggcca ctctactggg ttggccaaagg aacccttttgc tggccatct	720
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ggatctaaga ttttttctt catcagttca agcatcatca ctcatcagtc gtaagactcg	960
taacaaaata ttttttttca tataattaaat attatttccg catttaatgg atctacgttt	1020
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cttggttctt tgacaagaaaa aaaatataatt gtttttttgc ttctttttgt gttccaatct	1200
attttcgaga tttagacaag tgacacgtca tataccggat ttgttacctt gttaaagagt	1260
ttggggtaaa acaaatgttag aaaagttaaa ataaattgtg caataaaatgaa taaatacg	1320
tttatgttaa acaatgtatgt gaaaataaaa ttgaataatg gcagtgacca tggagttt	1380
tcagacatcc ctctgctgaa cagtgtggtt ggacacattt ttcatttcatc catctcgat	1440
ccttaccatg gttggtaagt catttattaa ctatccat gtaaaactatt agtactgtt	1500
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aatctttaga agccaatgaa aaagaatcca aaacttttt ttaatgata tgcgctatc	1740
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atgagtgaga ttgttaatc attatagaga caatttcatt ttcacaaaaa taaataaata	2100
cataactttt tataattggg gtttgagga gaataagcca tcggacacac caccagaacc	2160
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ctcttgtt ttatttat tttttttt cggaatttat ttctatgtct atgttcttag	2280
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tgtatctgtt agagcatttc caaaacaaaac ttataattt taaatataca gtttttttt	2460
ctctaaaaaa gaatttaaaa attttaaagt ttgagggacg aaacttcaaa tttgaacttt	2520
cactactcaa cttcaatattt gaaatttcat ctttttattt tacatttga tcattataat	2580
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gagatgcatt tagtggtt ggttagtaact cagaaaaatga aaaaatctata cttttatact	3300
ccctccgtt ttaatataa gtcgtttac agttatacac gtagattaag aaaaccatta	3360
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catagaaaaac cgaaaacgac atataatttga aacaaaaaaa atttctctaa aacgacttt	3540
ataaaaaaac ggagggagta gtacctaact ttaacgtatgg accacttata ttcgagtct	3600
tagcataaaa tgatttcctt cgtttttttt ttactttttt cattttttt tccctttcag	3660
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taaacacatc acatgcatac aattaatgcg aatacataac cagaatgaca aattttcaat	3780
gaatattttaa taccagtaag tactactccg taatgtat agtaatagtc atattaattt	3840
ttttttgtc atcaaacaaa cagtaatagt aatattaattt atttcagttg	3900

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89

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ggttggacgc tgcacgtac ttgcatcatc atggtcacga tgagaagttg cttggtaca 4440
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attnaaattaa atcattacgt cagtgacact ggtgatattg tcttctacga gacagatcca 4920
gatctctacg tttat 4935

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&lt;210&gt; SEQ\_ID NO 34

&lt;211&gt; LENGTH: 383

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Brassica napus

&lt;400&gt; SEQUENCE: 34

Met	Val	Val	Ala	Met	Asp	Gln	Arg	Ser	Asn	Val	Asn	Gly	Asp	Ser	Gly
1				5				10					15		

Ala	Arg	Lys	Glu	Glu	Gly	Phe	Asp	Pro	Ser	Glu	Gln	Pro	Pro	Phe	Lys
			20			25				30					

Ile	Gly	Asp	Ile	Arg	Ala	Ala	Ile	Pro	Lys	His	Cys	Trp	Val	Lys	Ser
35					40					45					

Pro	Leu	Arg	Ser	Met	Ser	Tyr	Val	Ala	Arg	Asp	Ile	Phe	Ala	Val	Ala
50				55			60								

Ala	Leu	Ala	Met	Ala	Ala	Val	Tyr	Phe	Asp	Ser	Trp	Phe	Leu	Trp	Pro
65					70			75			80				

Leu	Tyr	Trp	Val	Ala	Gln	Gly	Thr	Leu	Phe	Trp	Ala	Ile	Phe	Val	Leu
			85				90			95					

Gly	His	Asp	Cys	Gly	His	Gly	Ser	Phe	Ser	Asp	Ile	Pro	Leu	Leu	Asn
100					105					110					

Ser	Val	Val	Gly	His	Ile	Leu	His	Ser	Phe	Ile	Leu	Val	Pro	Tyr	His
115					120					125					

Gly	Trp	Arg	Ile	Ser	His	Arg	Thr	His	His	Gln	Asn	His	Gly	His	Val
130					135			140							

Glu	Asn	Asp	Glu	Ser	Trp	Val	Pro	Leu	Pro	Glu	Lys	Leu	Tyr	Lys	Asn
145					150			155		160					

Leu	Pro	His	Ser	Thr	Arg	Met	Leu	Arg	Tyr	Thr	Val	Pro	Leu	Pro	Met
165					170			175							

Leu	Ala	Tyr	Pro	Ile	Tyr	Leu	Trp	Tyr	Arg	Ser	Pro	Gly	Lys	Glu	Gly
180					185			190							

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Ser His Phe Asn Pro Tyr Ser Ser Leu Phe Ala Pro Ser Glu Arg Lys  
195 200 205

Leu Ile Ala Thr Ser Thr Cys Trp Ser Ile Met Leu Ala Thr Leu  
210 215 220

Val Tyr Leu Ser Phe Leu Val Gly Pro Val Thr Val Leu Lys Val Tyr  
225 230 235 240

Gly Val Pro Tyr Ile Ile Phe Val Met Trp Leu Asp Ala Val Thr Tyr  
245 250 255

Leu His His Gly His Asp Glu Lys Leu Pro Trp Tyr Arg Gly Lys  
260 265 270

Glu Trp Ser Tyr Leu Arg Gly Gly Leu Thr Thr Ile Asp Arg Asp Tyr  
275 280 285

Gly Ile Phe Asn Asn Ile His His Asp Ile Gly Thr His Val Ile His  
290 295 300

His Leu Phe Pro Gln Ile Pro His Tyr His Leu Val Asp Ala Thr Arg  
305 310 315 320

Ala Ala Lys His Val Leu Gly Arg Tyr Tyr Arg Glu Pro Lys Thr Ser  
325 330 335

Gly Ala Ile Pro Ile His Leu Val Glu Ser Leu Val Ala Ser Ile Lys  
340 345 350

Lys Asp His Tyr Val Ser Asp Thr Gly Asp Ile Val Phe Tyr Glu Thr  
355 360 365

Asp Pro Asp Leu Tyr Val Tyr Ala Ser Asp Lys Ser Lys Ile Asn  
370 375 380

What is claimed is:

1. A *Brassica napus* seed designated 1904 and represented by American Type Culture Collection (ATCC) Accession No. PTA-11273. 35
2. A plant grown from the seed of claim 1, wherein said seed comprises a modified allele at a fatty acid desaturase 3E fad3E locus. 40
3. A *Brassica napus* seed designated 2558 and represented by American Type Culture Collection (ATCC) Accession No. PTA-11274.
4. A plant grown from the seed of claim 3, wherein said seed comprises a modified allele at a fad3E locus. 45
5. The seed of the plant of claim 2, wherein said seed comprises a modified allele at a fad3E locus.
6. The seed of the plant of claim 4, wherein said seed comprises a modified allele at a fad3E locus.

\* \* \* \* \*